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**Short Communication**

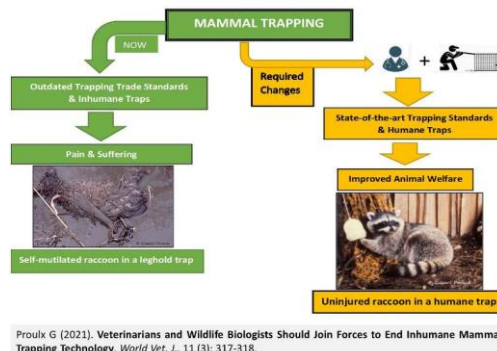
**Veterinarians and Wildlife Biologists Should Join Forces to End Inhumane Mammal Trapping Technology.**

Proulx G.

*World Vet. J.* 11(3): 317-318, 2021; pii:S232245682100043-11; DOI: <https://dx.doi.org/10.54203/scil.2021.wvj43>

**ABSTRACT:** Current mammal trapping standards uphold the use of inhumane trapping technology. For example, killing neck snares for the capture of canids, and rotating-jaw traps, and steel-jawed leghold traps for procyonids and mustelids, are being used by trappers despite decades of research showing that they are inhumane, and cause serious injuries and distress in captured animals. Many wildlife biologists unsuccessfully raised concerns about inhumane mammal trappings. This short communication stresses the need for veterinarians and wildlife biologists to work together to improve the fate of mammals captured in killing or restraining traps, and modify mammal trapping standards on the basis of animal welfare science.

**Keywords:** Humaneness, Mammal trapping, Traps, Trapping standards



Proulx G (2021). Veterinarians and Wildlife Biologists Should Join Forces to End Inhumane Mammal Trapping Technology. *World Vet. J.*, 11(3): 317-318.

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**Review**

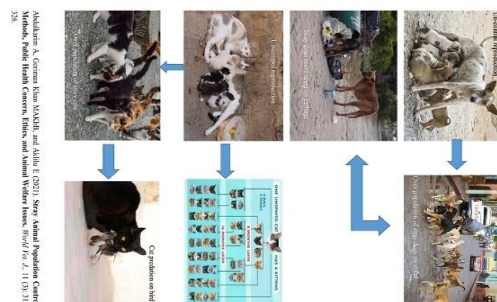
**Stray Animal Population Control: Methods, Public Health Concern, Ethics, and Animal Welfare Issues.**

Abdulkarim A, Goriman Khan MAKhB, and Aklilu E.

*World Vet. J.* 11(3): 319-326, 2021; pii:S232245682100044-11; DOI: <https://dx.doi.org/10.54203/scil.2021.wvj44>

**ABSTRACT:** Stray animal overpopulation has become one of the most serious global problems with many negative impacts on the community, environment, and public health. Most of the stray animals do not depend on humans for food and shelter, and therefore, can reproduce uncontrollably. The uncontrolled reproduction of stray animals increases their population that leads to a higher chance of predation, road traffic accidents, transmission of zoonotic diseases, and therefore, becoming vectors for some diseases. There are several methods for stray animal population control depending on the situation and the nature of the stray animals. These methods include contraceptives, trap-neuter-return, poisoning, euthanasia, and gun shooting among others. Each of the outlined methods has its advantages and disadvantages as to their practicality, ease of conducting, cost, effectiveness, ethics, and animal welfare issues. In conclusion, to achieve successful control measures of the stray animal population and the problem they create, the concerned authorities need to design and enact animal rights laws, provide medical care (treatment and vaccination), feeding, shelter for the animals, and control their reproduction. Public health and environmental agencies may improve the services by regulating personal and environmental hygiene, prevention, and control of zoonotic and transmissible diseases that can be transmitted from stray animals to the public and other livestock respectively.

**Keywords:** Animal welfare, Ethics, Population control methods, Public health, Stray animals



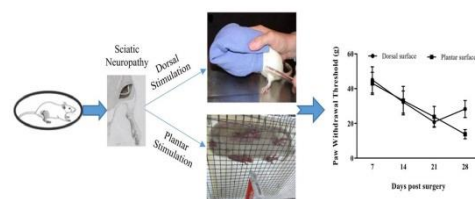
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**Research Paper**

**Comparison of Painful Response to Mechanical Stimulation of the Plantar and Dorsal Surface of Paw Following Chronic Constriction Injury-induced Neuropathic Pain.**

Ghanbari A and Mohammadi M.

**ABSTRACT:** Mechanical and thermal stimuli were used to evaluate neuropathic pain-like behavior in animal models usually. Mechanical stimulation of paw plantar surface is commonly used to determine mechanical allodynia. In the present study, paw withdrawal response to plantar surface stimulation was compared with paw withdrawal response to dorsal surface stimulation. To this end, a total of 30 female Wistar rats (180-220 g), were assigned randomly to three groups as intact (without any manipulation), sham (incision of skin and muscles without nerve injury), and neuropathy (sciatic nerve lesion) with 10 in each group. To induction of neuropathy (chronic constriction injury), four movable ligations were established around the sciatic nerve using catgut chromic suture with a distance of one millimeter apart and then wound incision was closed. In the sham group, the incision site was closed without nerve ligation. Mechanical allodynia was examined by Von Frey filaments for four weeks. The findings indicated that the paw withdrawal threshold following dorsal surface stimulation was significantly reduced compared to the sham group at day 21 post-surgery. Moreover, paw withdrawal threshold following plantar surface stimulation significantly decreased compared to the sham group at day 21 post-surgery. The present results regarding the sham group showed that the paw withdrawal threshold after mechanical stimulation of the plantar surface was not significantly different from that of the dorsal surface paw. In addition, and there was no significant difference between the paw withdrawal response to plantar surface and dorsal one. In conclusion, paw withdrawal threshold to plantar surface mechanical stimulation was not significantly different from one in dorsal surface following neuropathic pain induced by chronic constriction injury.



Ghanbari A and Mohammadi M (2021). Comparison of Painful Response to Mechanical Stimulation of the Plantar and Dorsal Surface of Paw Following Chronic Constriction Injury-induced Neuropathic Pain. *World Vet. J.*, 11 (3): 327-332.

**Keywords:** Mechanical allodynia, Neuropathic pain, Paw dorsal surface, Paw plantar surface, Rat

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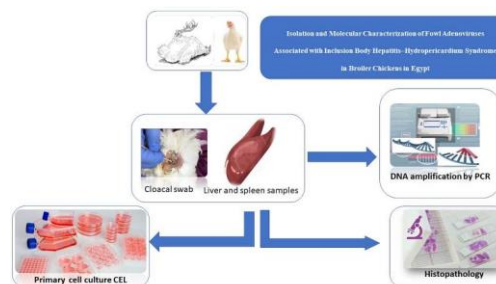
## Research Paper

### Isolation and Molecular Characterization of Fowl Adenoviruses Associated with Inclusion Body Hepatitis-hydropericardium Syndrome in Broiler Chickens in Egypt.

Al Naguib MG, Morsy EA, Samir M, Khattab MS, Khelifa DG, and Abdel-Alim GA.

World Vet. J. 11(3): 333-342, 2021; pii:S232245682100046-11; DOI: <https://dx.doi.org/10.54203/scil.2021.wvj46>

**ABSTRACT:** Avian adenoviruses are an extremely diversified group of pathogens that recently triggering a variety of problems for poultry production. In particular, Inclusion Body Hepatitis-Hydropericardium Syndrome (IBH-HPS), which has been observed in broiler birds from 3 to 6 weeks of age and is associated with anemia, hemorrhagic disorders, hydropericardium, and high mortality. The disease has been reported worldwide, and recently it was reported in many Egyptian farms, causing severe economic losses. Therefore, the current study aimed to isolate, and genetically type the most common Adenovirus serotypes associated with this syndrome in Egyptian farms. A total of 50 broiler chicken farms (3-6 weeks old) located in different Egyptian governorates were examined. Macroscopically, the diseased flock revealed hydropericardium, enlarged friable livers with ecchymotic hemorrhages, and varying mortality rates (1 to 7.5%). Histopathologically, severe diffuse necrotizing enteritis, hepatitis, pericarditis, and diffuse lymphoid depletion of the spleen were the most prominent lesions. Liver tissues and cloacal swabs were collected from all examined flocks for FAdVs detection by conventional polymerase chain reaction (PCR) targeting the L1 loop in the hexon gene. The PCR products were sequenced for typing of the detected viruses. It was found that 10 out of 50 flocks examined were PCR positive for FAdVs (20%). Phylogenetic analysis of the sequenced genes revealed that the obtained viruses clustered with reference strains belonging to FAdV type D and E serotype 2, 11, and 8a respectively. The isolation of both FAdV type D and FAdV type E were carried out on a primary cell culture chicken embryo liver cell (CEL) and the presence of these viruses was confirmed by PCR after the appearance of cytopathic effect (CPE). From this study, it could be concluded that both FAdVs types D and E are the most common adenoviruses circulating in poultry farms suffering from hydropericardium and inclusion body hepatitis.



Al Naguib MG, Morsy EA, Samir M, Khattab MS, Khelifa DG, and Abdel-Alim GA (2021) Isolation and Molecular Characterization of Fowl Adenoviruses Associated with Inclusion Body Hepatitis-hydropericardium Syndrome in Broiler Chickens in Egypt. *World Vet. J.*, 11 (3): 333-342.

**Keywords:** Broiler chicken, Chicken embryo liver cell, Fowl adenovirus, Hexon gene, Histopathology, Inclusion body hepatitis-hydropericardium syndrome, PCR

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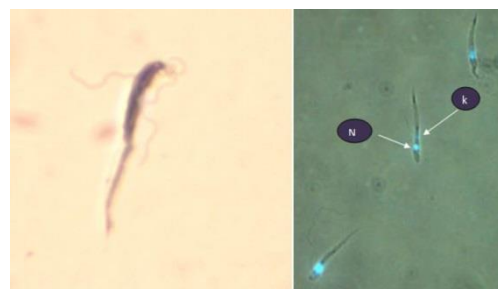
## Research Paper

### Molecular Identification of *Trypanosoma theileri* and Biology of Trypanosomes.

Fentahun T and Paeshuyse J.

World Vet. J. 11(3): 343-367, 2021; pii:S232245682100047-11; DOI: <https://dx.doi.org/10.54203/scil.2021.wvj47>

**ABSTRACT:** *Trypanosoma theileri* (*T. theileri*) is a non-pathogenic, cosmopolitan, and commensal protozoa of cattle. The main objective of the current study was to investigate the biology and feasibility of *T. theileri* as a model candidate for the discovery of a novel drug. In the present study, the isolates of *T. theileri* obtained from the Institute of Tropical Medicine (ITM) in SDM 79 were cultivated at 26°C. Eight experiments with different inoculum and different times were grown. The growth curve was plotted to check the growth trends. The doubling time in the logarithmic phase was determined to be 17.43 hours. In addition, an experimental infection was done on a 3-month-old Holstein Friesian calf to isolate the blood-streaming shape; however, it was not successful after the blood buffy coat smear and PBMC culture in RPMI 1640 and HMI 9. Furthermore, the viability was determined by quantitative colorimetric Resazurin assay in 96-well fluorescence Microplates containing 0.4 to 2.4 mM of Resazurin. On the other hand, the response to Pentamidine (1-100 ng/mL) showed a strong negative correlation between the fluorescence signal and the highest Pentamidine concentration. IC<sub>50</sub> was 9.25 ng/mL. Genomic DNA was extracted using the phenol-chloroform method. The gradient PCR amplification using *T. theileri* specific PCR (Tth625-PCR) primers was detected at 465 base pair (bp). In addition, the full-length 18S rDNA sequence was detected at 730 bp. In the silico analysis using common anti-trypanosome drug targets, no significant similarity could be found on either the DNA or the protein level. Nevertheless, homologous sequences have been identified among the drug targets for Ornithine decarboxylase. Therefore, the analysis might show the possibility of using *T. theileri* as a model for the search of new drugs once they have entire genome sequences. Analysis of the whole genome and transcriptome indicated a phylogenetic relationship between *T. theileri* and other pathogenic trypanosomes which can be the basis for novel drug development.



Fentahun T and Paeshuyse J (2021). Molecular Identification of *Trypanosoma theileri* and Biology of Trypanosomes. *World Vet. J.*, 11 (3): 343-367.

**Keywords:** Drug model, Novel drug, PCR, Resazurin, SDM 79, *Trypanosoma theileri*

[Full text-[PDF](#)] [[XML](#)] [[Google Scholar](#)]

## Research Paper

### Immunolocalization of Steroidogenic Enzymes (3 $\beta$ -hydroxysteroid dehydrogenase, 17 $\beta$ -hydroxysteroid dehydrogenase, and P450scc) in Rats with Testicular Dysfunction Treated with Mesenchymal Stem Cells-conditioned Medium.

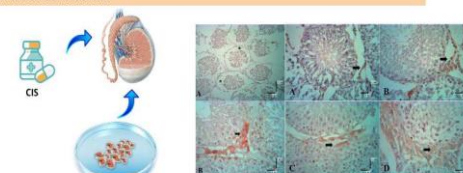
Khasanah LM, Budipitojo T, and Fibrianto YH.

*World Vet. J.* 11(3): 368-376, 2021; pii:S232245682100048-11; DOI: <https://dx.doi.org/10.54203/scil.2021.wvj48>

**ABSTRACT:** About 60-80 million couples in the world are suffering from infertility disease. Infertility is a major problem in patients coping with chemotherapy. The chemotherapy process can degenerate non-target organs, especially in testes. Infertility in male or testicular dysfunction is caused by the failure of proliferation and differentiation of the spermatogenic cells. Many studies reported that mesenchymal stem cells-conditioned medium promoted regenerative processes. The present study aimed to investigate the effect of mesenchymal stem cells-conditioned medium on the cisplatin-induced testicular dysfunction by examining the immunolocalization of steroidogenic enzymes, such as 3 $\beta$ -hydroxysteroid dehydrogenase, 17 $\beta$ -hydroxysteroid dehydrogenase, and P450scc which are considered as markers of steroid production. All experimental animals were divided into three groups, namely the control group, mesenchymal stem cells-conditioned medium treated group with an injection dose of 0.2 ml/kg body weight (BW, P1), and mesenchymal stem cells-conditioned medium treated group with an injection dose of 0.5 ml/kg BW (P2). Cisplatin was injected into both treated groups to induce testicular dysfunction. The testicular tissues were processed by the paraffin method, then cut to a thickness of 5  $\mu$ m, followed by immunohistochemical staining. The HSD3B1 immunoreactivities were found only in Leydig cells, and the intensity increased every week after the injection of mesenchymal stem cells-conditioned medium. The variety of weeks and groups was significantly different in the number of immunoreactive cells of HSD3B1. The results indicated a significant difference between one week after the first injection and the one week after the third and fourth injection. The findings showed a significant difference between the treated group with an injection dose of 0.2 ml/kg BW and the control group. The number of immunoreactive cells of HSD3B1 with an injection dose of 0.5 ml/kg BW was greater compared to the group that received an injection dose of 0.2 ml/kg BW. The intensity of HSD3B1 and HSD17B1 increased every week. The p450scc immunoreactive cells were only found in Leydig cells. The intensity of positive cells of p450scc in the treated group with an injection dose of 0.5 ml/kg BW was more intense, compared to the treated group with an injection dose of 0.2 ml/kg BW. The results of the current study showed that the injection of mesenchymal stem cells-conditioned medium can improve the regeneration of spermatogenic cells, and recover spermatogenesis proved by positive cells of HSD3B1, HSD17B1, and p450scc as markers of steroid production.

**Keywords:** Cisplatin, HSD17B1, HSD3B1, Mesenchymal stem cells-conditioned medium, P450scc, Testicular dysfunction

Immunolocalization of Steroidogenic Enzymes (3 $\beta$ -hydroxysteroid dehydrogenase, 17 $\beta$ -hydroxysteroid dehydrogenase, and P450scc) in Rats with Testicular Dysfunction Treated with Mesenchymal Stem Cells-conditioned Medium



Khasanah LM, Budipitojo T, and Fibrianto YH (2021). Immunolocalization of Steroidogenic Enzymes (3 $\beta$ -hydroxysteroid dehydrogenase, 17 $\beta$ -hydroxysteroid dehydrogenase, and P450scc) in Rats with Testicular Dysfunction Treated with Mesenchymal Stem Cells-conditioned Medium. *World Vet. J.* 11 (3): 368-376.

[Full text-[PDF](#)] [[XML](#)] [[Google Scholar](#)]

## Research Paper

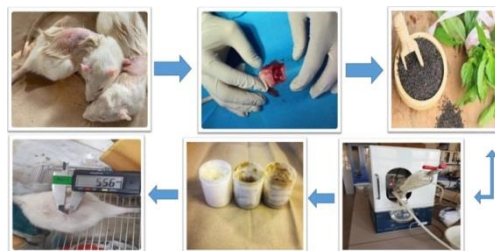
**The Influence of Basil Seed Hydroethanolic Extract on the Skin Wound Healing in Diabetic Male Rats.**

Hadi M, Moghtadaei-Khorasgani E, and Etesamnia MH.

World Vet. J. 11(3): 377-383, 2021; pii:S232245682100049-11; DOI: <https://dx.doi.org/10.54203/scil.2021.wvj49>

**ABSTRACT:** Diabetic wounds and *cutaneous* wounds are important issues in medical science. Basil is an herbaceous plant and has compounds such as terpenes, flavonoids, and antioxidant properties. A total of 50 male Wistar rats were allocated into 10 groups including the healthy group without treatment, the healthy group treated with 1% phenytoin, eucerin, 5% basil seed extract, 10% basil seed, diabetic group without treatment, 1% phenytoin, eucerin. After anesthesia of rats, we made a 4 cm<sup>2</sup> wound on the back of the animal, and different histopathological characteristics were examined, and also on days 3, 7, and 21, the area of wounds was analyzed. In the healthy group treated with 10% basil seed extract, it was revealed that the wound size was significantly healed in the research days. In the diabetic rat groups, the decrease in the wound area was not significant and there was no significant difference between groups. Histopathological findings on day 21 in the healthy group treated with 10% basil seed extract revealed proper epidermis formation and relatively dense dermis containing collagen filaments. While in the diabetic groups, healing was slower. The results indicated that basil extract with anti-inflammatory and antioxidant characteristics can quicken the repair of *cutaneous* wounds.

**Keywords:** Basil, Diabetes, Histopathology, Wound  
[Full text-[PDF](#)] [XML] [Google Scholar]



The freshly prepared samples were first washed with saline and placed in 10% formalin for 48 hours. Then, the samples were dehydrated in a tissue processor with alcohols of 70, 80, 95% and absolute in each for one hour. In the next step, each sample was clarified with xylol for 45 minutes. Impregnation with molten paraffin was then done in a container for 2 and 3 hours. In the next step, the samples were embedded. The incision was made with a thickness of 3 microns by microtome and the incisions were placed in a container of hot water. Incubation was then performed at 65 °C to remove paraffin. In the next step, staining was done. In such a way that the first, second and third dishes were 5 minutes each. The steps were first absolute alcohol, 95, 90, 70 and distilled water, respectively, then staining hematoxylin in the next stage after washing with running water. Then impregnate with alcohol acid, rinse with running water and stain with eosin dye to stain the cytoplasm. Then it goes through the stages of alcohol 70, 80, 95% and absolute alcohol and it is clarified again with xylol and then it is mounted.

## Research Paper

**Concurrent Respiratory Disease in Broiler Chickens in Egypt during 2020.**

Yehia N, Amer F, Samir A, Samy M, Sedeek A, Rebie N, Mohammed W, and Hagag N.

World Vet. J. 11(3): 384-394, 2021; pii:S232245682100050-11; DOI: <https://dx.doi.org/10.54203/scil.2021.wvj50>

**ABSTRACT:** Poultry production has been affected by multiple respiratory diseases triggering serious economic losses in Egypt. The current study aimed to investigate the situation and genetic evolution of respiratory diseases in Egypt during 2020. A total of 53 samples were collected from infected flocks suffering from respiratory signs and variable mortality rates from nine governorates in Egypt during 2020. The collected samples were examined for the detection of respiratory disease viruses (Avian influenza virus (AIV (H5N8, H9N2), Infectious bronchitis virus (IBV), and Newcastle disease virus (NDV)) by rRT-PCR. The single infection was confirmed in 90.6% (37.7% I.B, 30.2% AIV (H5N8), 9.4% I.B and 5.7% NDV) and co-infection of HPAIV (H5N8) + I.BV and LPAIV (H9N2) +IBV were detected in 3.8% of nine governorates. The HA gene of HPAIV (H5N8) was cluster to clad 2.3.4.4.1b in a new branch with characteristic specific mutations especially in T140A in antigenic site A and R72S in the receptor-binding site, compared to A/duck/Egypt/F446/2017 with low A.A identity percent with vaccinal strains of H5N1 and H5N2 reaching to 91.9-94% and 84.6%, respectively. The HA gene of AIV (H9N2) belonged to A/quail/Hong Kong/G1/97-like virus clustered with group B with a specific mutation (212I) that may affect the human transmission of the virus. The HVRs of S1 gene of IBV cluster to GI23 (Egy Var I) clad with multiple mutations in HVR1 and HVR2, compared to IBV/CU/4/2014 and low identity percent (68.3-78.8%) with vaccine strains (H120, M41, 4/91). In conclusion, respiratory disease continues to circulate and rapidly evolve in Egypt during 2020.

**Keywords:** HPAIV (H5N8), IBV, Genetic characterization, LPAIV(H9N2), Respiratory disease



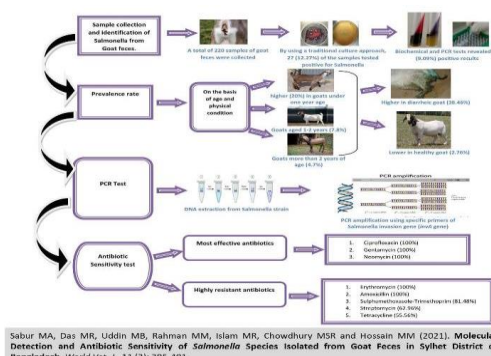
Yehia N, Amer F, Samir A, Samy M, Sedeek A, Rebie N, Mohammed W, and Hagag N (2021). Concurrent Respiratory Disease in Broiler Chickens in Egypt during 2020. World Vet. J., 11 (3): 384-394.

[Full text-[PDF](#)] [XML] [Google Scholar]

## Research Paper

**Molecular Detection and Antibiotic Sensitivity of Salmonella Species Isolated from Goat Feces in Sylhet District of Bangladesh.**

Sabur MA, Das MR, Uddin MB, Rahman MM, Islam MR, Chowdhury MSR and Hossain MM.

World Vet. J. 11(3): 395-401, 2021; pii:S232245682100051-11; DOI: <https://dx.doi.org/10.54203/scil.2021.wvj51>

Sabur MA, Das MR, Uddin MB, Rahman MM, Islam MR, Chowdhury MSR and Hossain MM (2021). Molecular Detection and Antibiotic Sensitivity of Salmonella Species Isolated from Goat Feces in Sylhet District of Bangladesh. World Vet. J., 11 (3): 395-401.



**ABSTRACT:** The present study aimed at the molecular detection of *Salmonella* species from feces of goats and the characterization of the isolated *Salmonella* by biochemical and antimicrobial sensitivity techniques. A total of 220 goat feces samples were collected, of which 27 (12.27%) were positive for *Salmonella* by conventional culture methods and 20 (9.09%) by biochemical and PCR techniques. The prevalence was higher in goats under one year of age (20%), compared to older animals aged one to two years (7.8%) and more than two years of age (4.7%), respectively. Moreover, the prevalence of diarrheic goats was significantly higher (38.46%) than healthy animals (2.76%). DNA was extracted from *Salmonella* strains and amplified by PCR using the specific primers of *Salmonella* invasion gene (*invA* gene). The antibiotic sensitivity test indicated that Ciprofloxacin (100 percent sensitivity), Gentamycin (100 percent sensitivity), and Neomycin (100 percent sensitivity) were the most effective antibiotics for the majority of *Salmonella* isolates. On the other hand, *Salmonella* isolates were found to have substantially high resistance to Erythromycin (100%), Amoxicillin (100%), Trimethoprim-Sulfamethoxazole (81.48%), Streptomycin (62.96%), and Tetracycline (55.56 percent). Since the rate of *Salmonella* carriers was relatively high, eating goat meat could increase the risk of foodborne salmonellosis.

**Keywords:** Antibiotic sensitivity, Goat isolation, PCR detection, *Salmonella*

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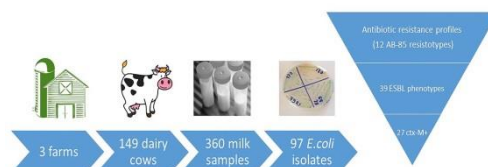
## Research Paper

### Antibiotic Resistance Profile of *Escherichia coli* Isolated from Bovine Subclinical Mastitis of Dairy Farms in Algeria from 2017 to 2019.

Ghallache L, Mohamed-Cherif A, China B, Mebkhouf F, Boilattabi N, Bouchemal A, Rebia A, Ayachi A, Khelef D, Miroud K, and Ait-Oudhia Kh.

World Vet. J. 11(3): 402-415, 2021; pii:S232245682100052-11; DOI: <https://dx.doi.org/10.54203/scil.2021.wvj52>

**ABSTRACT:** Mastitis in cows is a major problem in dairy farms leading to a decrease in the quantity and quality of milk. The aim of the present study was to examine the association between the presence of *Escherichia coli* (*E. coli*) in milk and the subclinical mastitis, and to characterize the antibiotic resistance profiles of the isolated *E. coli*. In the current study, a total of 360 cow raw milk samples from three dairy farms of the region of Algiers were analyzed. The analysis period lasted from Spring 2017 to Winter 2019. The California Mastitis Test (CMT) was applied to detect subclinical mastitis. The *E. coli* strains were isolated from milk using conventional bacteriological methods. The antibiotic resistance profile of the isolated *E. coli* strains to 12 different antibiotics was tested using the disk diffusion method. On  $\beta$ -lactamase-producing strains, a double diffusion test was applied to identify the Extended-spectrum  $\beta$ -lactamase (ESBL) phenotype. Finally, the *ctXx-M* genes were amplified by PCR. Two-thirds (66.4%) of the milk samples were positive for the CMT test. A total of 97 *E. coli* strains were isolated from the milk samples, their resistance to antibiotics was tested, and 3.1% of the strains were resistant to trimethoprim-sulfamethoxazole, 6.2% to chloramphenicol, 12.3% to gentamicin, 13.4% to colistin, 23.3% to amoxicillin/clavulanate, 31.9% to kanamycin, 39.2% to enrofloxacin, 51.5% to cefotaxime, 52% to tetracycline, 57.7% to ampicillin, 74.3% to nalidixic acid, and 75.3% to amoxicillin. Furthermore, most of the *E. coli* strains (92.8%) were resistant to more than one antibiotic with a Multiple Antibiotic Resistance index ranging from 0 to 0.8. The 50 strains resistant to cefotaxime were analyzed for an ESBL phenotype. 39 of them (78%) were positive to the double-disk synergy test. Among the 39 ESBL positive strains, 27 (69.2%) were confirmed for the presence of a CTX-M gene by PCR. The present study showed that multiple drug-resistant *E. coli*, including ESBL-carriers, were frequently isolated from the milk of dairy cows in Algeria. The results underlined that the use of antibiotics on farms must be reasoned to avoid the spread of resistant strains in animals and human populations.



Ghallache L, Mohamed-Cherif A, China B, Mebkhouf F, Boilattabi N, Bouchemal A, Rebia A, Ayachi A, Khelef D, Miroud K, and Ait-Oudhia Kh (2021). Antibiotic Resistance Profile of *Escherichia coli* Isolated from Bovine Subclinical Mastitis of Dairy Farms in Algeria from 2017 to 2019. World Vet. J., 11 (3): 402-415.

**Keywords:** Antibiotic Resistance, Cows, CTX-M gene, *Escherichia coli*, Milk, Subclinical Mastitis

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## Research Paper

### Effect of Larvicidal Extract N-Hexane Lime Leaves (*Citrus hystrix*) on Larva Instar III Mosquito (*Culex quinquefasciatus*)

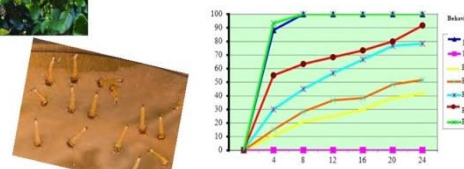
Hanif M, Lastuti NDR, and Kurnijasanti R.

World Vet. J. 11(3): 416-421, 2021; pii:S232245682100053-11; DOI: <https://dx.doi.org/10.54203/scil.2021.wvj53>

**ABSTRACT:** *Culex quinquefasciatus* mosquitoes are a vector of transmission of several types of diseases, such as filariasis, Japanese encephalitis, and dirofilariasis. Larval control is the key strategy of disease control programs caused by vectors around the world because it can prevent larvae to enter the adult stage. Control of mosquito larvae that are often used is chemically controlled. *Citrus hystrix* is a natural plant and contains chemical compounds that have biological activity, such as flavonoids, carotenoids, and limonoids. The present study aimed to determine the effect of citrus leaf extract (*Citrus hystrix*) on the



Effect of Lime Leaves (*Citrus hystrix*) on Larva Instar III Mosquito



Hanif M, Lastuti NDR, and Kurnijasanti R (2021). Effect of Larvicidal Extract N-Hexane Lime Leaves (*Citrus hystrix*) on Larva Instar III Mosquito (*Culex quinquefasciatus*). World Vet. J., 11 (3): 416-421.

mortality of the larva *Culex quinquefasciatus*. The method used in the current study was a laboratory experimental study method with the experimental design using a completely randomized design. The research plot carried out was the rearing larvae of *Culex quinquefasciatus*, the manufacture of citrus leaf extract, the manufacture of larvicidal material, and the observation phase after treatment. Extract N-hexane *omplet name hystrix* leaf has high toxicity because it contains essential oils, flavonoids, alkaloids, terpenoids, saponins, and Limonoids. The Optimal concentration of N-hexane extracts of citrus leaves that lead to larval mortality of *Culex quinquefasciatus* in vitro amounted to 93.33% on 4 hours of observation with a concentration of 4000 ppm. Extract N-hexane lime leaf has a larvicidal effect on the larva *Culex quinquefasciatus* in vitro.

**Keywords:** *Citrus hystrix*, *Culex quiquefasciatus*, Extract, Mosquitoes, Larvicide

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## Research Paper

### Effect of Processed Lemon Pulp with *Saccharomyces cerevisiae* in Zaribi Goats Diet on Milk Production, Nutrients Digestibility, Blood Parameters, and Reproductive Performance

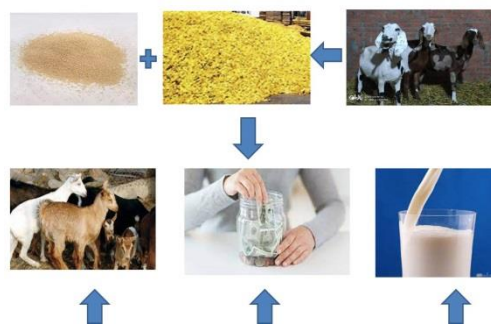
Sayed HAE, El-Maghraby MM, and Elbadawy MM.

*World Vet. J.* 11(3): 422-430, 2021; pii:S232245682100054-11; DOI: <https://dx.doi.org/10.54203/scil.2021.wvj54>

**ABSTRACT:** The aim of the present study was to evaluate the effect of replacing concentrate feed (CFM) mixture with different levels of treated lemon pulp (TLP) on milk production and composition, nutrients digestibility, and blood parameters of Zaribi goats. A total of 24 Zaribi female goats at the end of the pregnant period were divided into three groups of eight according to live weight. Concentrate feed mixture and clover hay at a rate of 50:50 were offered twice a day. Treated lemon pulp replaced CFM at rates 0%, 25%, and 50 % in diets of R1, R2, and R3 treatment groups, respectively. The feeding trial lasted 90 days. Dry matter digestibility and Nitrogen free extract digestibility were significantly raised by the increased level of TLP in groups R2 (72.37% and 70.36%) and R3 (72.28% and 70.30%), compared to (70.99% and 68.51%) in R1, respectively. The same trend was observed for organic matter digestibility (OMD), crude protein digestibility (CPD), crude fiber digestibility (CFD), and ether extract digestibility (EED) with R3, compared with either R1 or R2. However, there were insignificant differences between R1 and R2 in terms of OMD, CPD, and EED but CFD was significantly higher in R2, compared to R1. There was a significant increase in the milk yield by the increased level of TLP in the diet and for R2 (1448.4g/h/d) and R3(1558.7g/h/d), while it was reported as 1377.6 g/h/d in the control group. Feeding dams on the R3 diet had a significant effect on improving total antioxidant capacity by 63.5%, compared with control. The results of the present study indicated that the replacement of CFM by TLP decreased the costs of feed and increase milk production for the replacement level by 50% (R3). Moreover, no adverse effects were noticed on nutrients digestibility and blood parameters of the investigated samples.

**Keywords:** Blood parameters, Lemon pulp, Milk production, *Saccharomyces cerevisiae*

Sayed HAE, El-Maghraby MM, and Elbadawy MM (2021). Effect of Processed Lemon Pulp with *Saccharomyces cerevisiae* in Zaribi Goats Diet on Milk Production, Nutrients Digestibility, Blood Parameters, and Reproductive Performance. *World Vet. J.*, 11 (3): 422-430.



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## Short Communication

### The Effects of Different Concentrations of Bay Leaf Extract on Shelf Life of Preserved Scad Fish

Fuaidah P, Triastuti J, and Pramono H.

*World Vet. J.* 11(3): 431-438, 2021; pii:S232245682100055-11; DOI: <https://dx.doi.org/10.54203/scil.2021.wvj55>

**ABSTRACT:** Scad fish (*Decapterus kurroides*) is the most productive fish species. To provide the added value, fishermen can process scad fish into various forms of processed products, such as preserved fish, which extend the shelf life. In order to extend the shelf life of canned fish, natural preservatives could be used that contain antimicrobial agents, such as bay leaves. The present study aimed to determine the effects of bay leaf extract in different concentrations on the shelf life of preserved scad fish. In the present study, the completely randomized design was used as an experimental research method. The treatments given differed in terms of the concentration of the bay leaf extract. The groups were treated as the scad fish without bay leaf extract (A), scad fish soaked in 6% of bay leaf extract (B), scad fish soaked in 7% of bay leaf extract (C), scad fish soaked in 8% of bay leaf extract (D), and scad fish soaked in 9% of bay leaf extract (E). The analyzed parameters included the total plate count, pH, and water level test using Analysis of Variance (ANOVA). The supportive parameters observed included an organoleptic test. The results indicated that the lowest total bacterial count from the beginning to the end of the experiment (18 hours) occurred on treatment C



Fuaidah P, Triastuti J, and Pramono H (2021). The Effects of Different Concentrations of Bay Leaf Extract on Shelf Life of Preserved Scad Fish. *World Vet. J.*, 11 (3): 431-438.

(7% of bay leaf extract addition), which was  $1.54 \times 10^3$  to  $5.85 \times 10^6$ . Regarding the water level test from the beginning to the end of the experiment, treatment A (control) was not significantly different from other treatments. The difference in the concentration of bay leaf extract on scad fish effectively inhibited the growth of bacterial colonies. In conclusion, adding bay leaf extract to scad fish could inhibit bacteria for up to 12 hours. Treatment C (7% of bay leaf extract) gave the best results since this concentration level could inhibit the bacteria on scad fish.

**Keywords:** Bay leaf, Preservation, Scad fish, Shelf life

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## Research Paper

### Effects of Laying Cycle Periods on Egg Quality, Egg Chemical Composition, and Reproductive Performance of Japanese Quail Breeders Reared in Northern Algeria

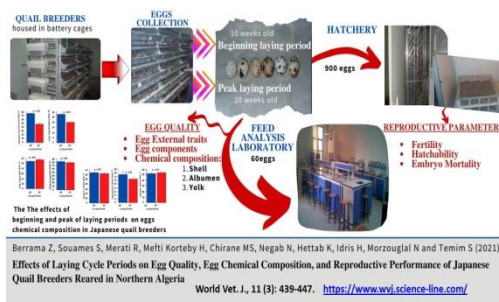
Berrama Z, Souames S, Merati R, Korteby HM, Chirane MS, Negab N, Hettab K, Idris H, Morzouglal N, and Temim S.

World Vet. J. 11(3): 439-447, 2021; pii:S232245682100056-11; DOI: <https://dx.doi.org/10.54203/scil.2021.wvj56>

**ABSTRACT:** Egg quality traits in quail breeders depend on various factors which may influence embryo survival during incubation, affecting the chicks' production and quality. The current study aimed to determine the effect of the laying period on the external and internal quality of the egg, the chemical composition of albumen, yolk, and eggshell as well as the reproductive parameters of quail breeders. A total of 450 quails (*Coturnix japonica*) obtained from the same hatchery, were reared in a battery cage with a sex ratio of 1 male to 3 females. All the quails were subjected to standard breeding conditions and fed a balanced laying diet. A total of 960 eggs were collected at two different periods of the laying cycle, the beginning period of the laying (BP indicating 10 weeks of quail age) and the peak period of laying (PP showing 20 weeks of quail age).

At each laying period, 30 eggs were used to analyze the various parameters of egg quality, and 450 eggs were randomly selected to assess the reproductive performances. Overall, no differences in the external quality of eggs, such as egg length, egg width, and the egg shape index, were recorded between the two laying periods. Apart from albumen weight that tended to be higher at the peak laying period, the shell and the yolk weights were not affected by the period of laying. Additionally, Japanese quail tend to deposit similar proportions of shell, albumen, and yolk at the two periods of laying. Likewise, the rate of dry matter of the three egg components, shell mineral concentrations, and yolk fat concentrations did not show any noticeable variation with the laying period. The most significant effect of the laying period was related to the potential reduction in the total protein content of the albumen and the yolk of eggs laid at the peak period of laying. Finally, the laying period did not significantly affect the fertility and hatchability rate of the incubated quail eggs but slightly improved the embryonic mortality rate during the peak laying phase.

**Keywords:** Albumen, *Coturnix japonica*, Eggshell, Fertility, Quail



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## Research Paper

### The Role of Salinity in Histopathology Description of Jatim Bulan Tilapia Juvenile (*Oreochromis niloticus*) Exposed by Lead (Pb)

Batista FR, Triastuti J, and Pursetyo KT.

World Vet. J. 11(3): 448-455, 2021; pii:S232245682100057-11; DOI: <https://dx.doi.org/10.54203/scil.2021.wvj57>

**ABSTRACT:** The decrease in the number of lands for aquaculture will cause the freshwater fish aquaculture, especially tilapia is reared. As one of the efforts to survive, the breeders use the coastal area to anticipate, so the freshwater fish can adapt to the sea waters. Heavy metal pollution near the coasts (or in the coastal waters) has great potential impacts on the environment. The present study aimed to find out the effect of Lead (Pb, heavy-metal) exposure on the Jatimbulan Juvenile tilapia with the different salinity towards histopathology description of the gill, intestine, and the skin tissues. The method used was an experimental method with a completely randomized design using six treatments and three times repetition. The observed parameters included the changes in histopathology description of gill, intestine, and skin organs of Jatimbulan juvenile tilapia. Kruskal-Wallis scoring method was used for data analysis and was continued by Mann-Whitney. The result of the current study showed the effect of salinity towards histopathology description of gill, intestine, and skin of Jatimbulan juvenile tilapia. The effect of changes was proven with the histopathology description in the form of the damage of edema, hyperplasia, and necrosis on the gill tissue; the damage of edema, atrophy, and necrosis on the gill tissue as well as the damage of edema, atrophy, hemorrhagic and necrosis on the gill tissue. From the result of the current study which has been conducted, it can be concluded that the heavy-metal exposure by lead was 0.03 ppm on the salinity of 10 ppt and 20 ppt. given the significant effect on the histopathology description of gill, intestine, and skin of Jatimbulan juvenile tilapia.

**Keywords:** Histopathology, Lead, Metal, *Oreochromis niloticus*, Salinity



Histopathology Description of Jatim Bulan Tilapia Juvenile

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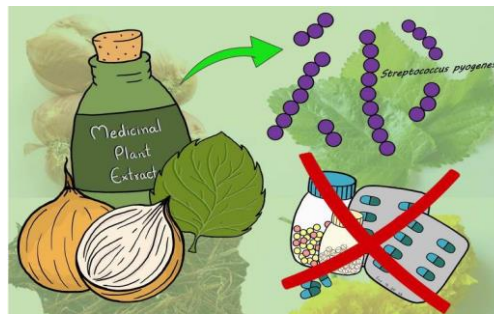
## Research Paper

### **In Vitro Antibiotic Activity of red Shallot (*Allium ascalonicum*), Mulberry (*Morus indica*), and Marigold (*Tagetes erecta*) Extracts against *Streptococcus pyogenes***

Mekvimol T, Chaipunna Ch, Poonthong G, and Pumipuntu N.

World Vet. J. 11(3): 456-461, 2021; pii:S232245682100058-11; DOI: <https://dx.doi.org/10.54203/scil.2021.wvj58>

**ABSTRACT:** Bacterial infection is a major global health concern. One of the critical problems is the widespread of antimicrobial-resistant bacteria from inappropriate and prolonged use of antimicrobial agents in both humans and animals. Plant extracts might afford the chance to replace antibiotic drugs and reduce the emerging of antimicrobial-resistant bacteria. This study aimed to examine the antibiotic activity of ethanolic crude extracts of some Thai medicinal plants grouping in their parts as whole onions of red shallot (*Allium ascalonicum*), petals of marigold (*Tagetes erecta*), mulberry leaves, and root barks (*Morus indica*) to inhibit the growth of *Streptococcus pyogenes*. The antibiotic activities of the crude extract of three Thai medicinal plants using absolute ethanol were trialed against *Streptococcus pyogenes* using the disk diffusion method. Erythromycin and Ceftriaxone discs were chosen to be positive control standards as the representative of antibiotic drugs. Each dried plant extracts was prepared to test the inhibition with a concentration at 25, 50, and 75 mg/mL stock solution. The results showed that three groups from all testing groups of Thai medicinal plant extracts had the potential of antibiotic activity against *S. pyogenes*. The highest antibiotic activity against *S. pyogenes* was detected from whole onion extract red shallot followed by the extract of the mulberry leaves and root barks of mulberry strain Nakhon Ratchasima 60 (Nak 60) while the extract of marigold petal did not present antibiotic activity. The results revealed that crude extract of those two Thai medicinal plants, including red shallot and mulberry, had antibiotic activity against bacterial growth of *S. pyogenes* in the experiment and these medical Thai plants had potential benefits for developing as alternative treatment agents for *S. pyogenes* infections in both humans and animals in the future.



Mekvimol T, Chaipunna Ch, Poonthong G, and Pumipuntu N (2021). In Vitro Antibiotic Activity of Red Shallot (*Allium ascalonicum*), Mulberry (*Morus indica*), and Marigold (*Tagetes erecta*) Extracts against *Streptococcus pyogenes*. World Vet. J., 11 (3): 456-461.

**Keywords:** Antibiotic activity, Ethanolic crude extract, Thai medicinal plants, *Streptococcus pyogenes*

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## Research Paper

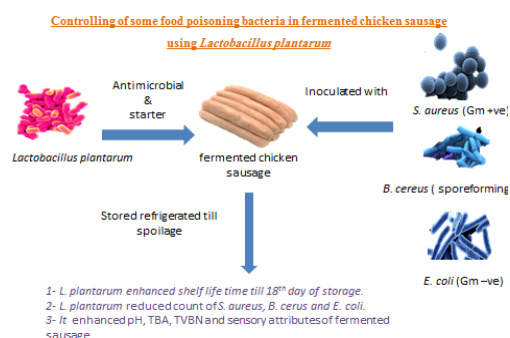
### **Controlling Food Poisoning Bacteria in Fermented Chicken Sausage Using *Lactobacillus plantarum***

Elsabagh R, Nada ShM, and Abd-Elaaty EM.

World Vet. J. 11(3): 462-468, 2021; pii:S232245682100059-11; DOI: <https://dx.doi.org/10.54203/scil.2021.wvj59>

**ABSTRACT:** *Lactobacillus plantarum* (*L. plantarum*) bacteria is generally recognized as safe and widely used in the food industry. The current study aimed to study the antimicrobial effects of *L. plantarum* against some food poisoning microorganisms, such as *Staphylococcus aureus* (*S. aureus*), *Bacillus cereus* (*B. cereus*), and *Escherichia coli* (*E. coli*) in oriental fermented chicken sausage for 18 days of storage at 4°C. The *L. plantarum* has broad-spectrum antimicrobial effects that enhance the quality and safety of food products. *L. plantarum* reduced the count of *S. aureus*, *B. cereus*, and *E. coli* to 1.54, 4.26, and 3.03 Log<sub>10</sub>, respectively, after 18 days of refrigerated storage. Moreover, there were significant effects of *L. plantarum* on pH, thiobarbituric acid, total volatile basic nitrogen, and sensory attributes of fermented sausage samples during storage time. It was revealed that *L. plantarum* enhanced the physico-chemical, sensory attributes, and shelf life of fermented chicken sausage. Moreover, *L. plantarum* inhibited the inoculated food poisoning bacteria in fermented chicken sausage. In conclusion, it is recommended to use *L. plantarum* in fermented meat products as a starter and a bio-preservative to enhance the quality of the fermented chicken sausage.

**Keywords:** Chicken sausage, Food safety, *Lactobacillus plantarum*, Probiotic



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## Short Communication

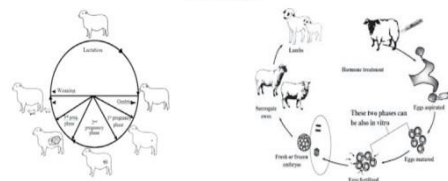
### **The Effect of the Different Artificial Insemination Time Periods on the Pregnancy Rate of Sapudi Ewes**

Putri CD, Ismudiono, and Poetranto ED.

**ABSTRACT:** Brucellosis Artificial insemination is required to increase the reproduction rate in ruminant breeding. The artificial insemination success rate in sheep only reaches 47.6%, whereas the proposed ideal rate is 70%. One of the factors influencing the artificial insemination success rates in sheep is improper estrus detection, resulting in no fertilization. The present study aimed to determine the effect of different artificial insemination time periods on the pregnancy rates of Sapudi ewes. The research design was based on a completely randomized design. A total of 20 female Sapudi sheep were divided into four treatment groups with five repetitions (for each group). In addition, the observed variables were artificial insemination time in Sapudi ewes. Estrus synchronization in ewes was conducted by injecting PGF2 $\alpha$ . The results of the research indicated that ewes subjected to artificial insemination 6, 12, 18, and 24 hours after estrus had a pregnancy rate of 20%, 100%, 60%, and, 60%, respectively. It can be concluded that the time differences in artificial insemination significantly influence the pregnancy rate in Sapudi ewes'.

**Keywords:** Artificial insemination, Estrus, Pregnancy rate, Sapudi ewes

Effect of Artificial Insemination Time Periods on the Pregnancy Rate of Sapudi Ewes



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## Research Paper

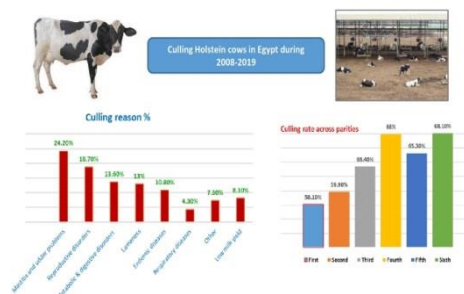
### Milk Production and Reproductive Performance of Retained and Culled Cows in a Large Holstein Herd in Egypt

Fahim NH, Ibrahim MA-AM, Amin AH, and Sadek RR.

World Vet. J. 11(3): 474-483, 2021; pii:S232245682100061-11; DOI: <https://dx.doi.org/10.54203/scil.2021.wvj61>

**ABSTRACT:** The study aimed to identify the culling reasons of Holstein cows raised in a large commercial herd in Egypt with emphasis on the performance of retained and culled cows. A total of 31534 complete lactation records for 10994 cows calved from 2008 to 2019 were used. The overall rate of culling per lactation was 61.1%. Involuntary culling represented 92% of all culling cases. The reasons for culling included mastitis and udder problems (24.2%), reproductive disorders (18.7%), metabolic and digestive disorders (13.6%), lameness (13%), endemic diseases (10.8%), low milk yield (8.1%), respiratory diseases (4.3%) and unknown causes (7.3%). Means of 305-day milk yield and daily milk yield were significantly lower in culled cows than the retained ones. On the other hand, no significant differences were observed between culled and retained cows for days open and the number of services/conception. The high involuntary culling rate of Holstein under the Egyptian conditions revealed that management practices regarding mastitis prevention and reproductive efficiency should be improved.

**Keywords:** Culling reasons, Egypt, Holstein, Milk production, Reproductive performance



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## Short Communication

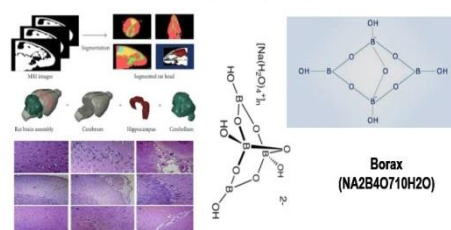
### The Effects of Borax (Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>·10H<sub>2</sub>O) on Histopathology of Wistar Rats' Cerebrum (*Rattus norvegicus*)

Elfan F, Kuncorojakti S, and Triakoso N.

World Vet. J. 11(3): 484-488, 2021; pii:S232245682100062-11; DOI: <https://dx.doi.org/10.54203/scil.2021.wvj62>

**ABSTRACT:** The present study aimed to determine the effects of borax (Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>·10H<sub>2</sub>O) addition on the changes of histological cerebrum imaging in the brains of white mice (*Rattus norvegicus*). The current research was an experimental study with randomization of 24 white mice that were divided into four treatment groups with five replications. Borax was dissolved for each treatment with a dose of 19 mg/mouse/day, 26 mg/mouse/day, and 37 mg/mouse/day, and it was administered orally for 14 days. Then, it was analyzed statistically using the Kruskal-Wallis test. The statistical analysis results suggested that there were significantly different results in each treatment group. The control treatment with an administration dose of 26 mg/rat/day had a significantly different result in the worst cloudy swelling degeneration of cerebrum in histopathology imaging on Wistar rats (*Rattus norvegicus*). Using the Mann-Whitney test, it was found that the dose of borax at 37 mg/rat/day led to significant difference, compared to the other treatment groups, which means that 37 mg/rat/day of borax caused the worst pyramidal cell necrosis in histopathology imaging of the cerebrum on white

Effects of Borax (NA2B4O7·10H2O) on Histopathology of Wistar Rats' Cerebrum



mice. Borax exposure on Wistar rats (*Rattus norvegicus*) can cause cloudy swelling at a dose of 26mg/head/day, and pyramidal cell necrosis at a dose of 37 mg/head/day.

**Keywords:** Borax, Cerebrum, Cloudy swelling, Necrosis

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## Research Paper

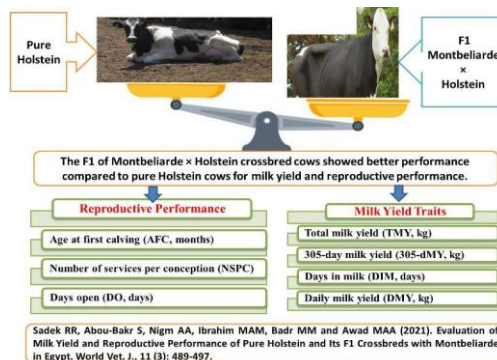
### Evaluation of Milk Yield and Reproductive Performance of Pure Holstein and Its F1 Crossbreds with Montbeliarde in Egypt

Sadek RR, Abou-Bakr S, Nigm AA, Ibrahim MAM, Badr MM and Awad MAA.

*World Vet. J.* 11(3): 489-497, 2021; pii:S232245682100063-11; DOI: <https://dx.doi.org/10.54203/scil.2021.wvj63>

**ABSTRACT:** The present study was carried out to compare the milk yield and reproductive performance of pure Holstein (HO) cows with those of their first generation (F1) crossbreds with Montbeliarde cows (MO) in four commercial dairy herds under Egyptian conditions. Data used in the current study comprised 2268 records for the first four lactations of 531 HO and 536 MO × HO F1 crossbred cows during the period between 2012 and 2020. Data were analyzed using the least squares method by XLSTAT software. The MO × HO crossbred cows were significantly superior compared with pure HO cows for 305-day milk yield, scoring 9210 ± 96 kg versus 7987 ± 149 kg. Moreover, MO × HO F1 crossbred cows had a significantly higher daily milk yield (30.0 ± 0.45 kg) than pure HO cows (25.9 ± 0.52 kg). However, pure HO cows had significantly greater days in milk (399 ± 6 days) than MO × HO crossbred cows (341 ± 5.2 days). With regard to reproductive performance, MO × HO F1 crossbred cows had significantly less number of services per conception and days open than pure HO cows (2.6 ± 0.16 vs. 3.7 ± 0.18) and (132 ± 5.2 days vs. 190 ± 6 days), respectively. However, the statistical difference between MO × HO F1 crossbred cows and pure HO cows for age at first calving was not significant (22.9 ± 0.11 vs. 23.1 ± 0.15 months, respectively). It can be concluded that under Egyptian subtropical conditions, the first generation of MO × HO crossbred cows exhibit better performance, compared to pure HO cows in milk yield and reproductive traits. These findings could provide an effective strategic option for the genetic improvement of dairy cattle in hot subtropical regions.

**Keywords:** Crossbreeding, Egypt, Holstein, Milk Yield, Montbeliarde, Reproduction



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## Short Communication

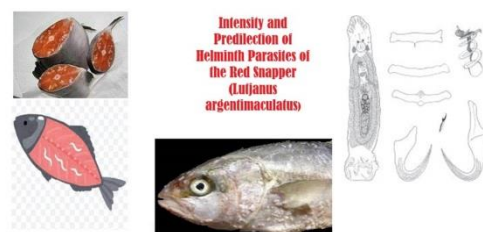
### Intensity and Predilection of Helminth Parasites of the Red Snapper (*Lutjanus argentimaculatus*)

Prasetya AW, Kismiyati, and Subekti S.

*World Vet. J.* 11(3): 498-503, 2021; pii:S232245682100064-11; DOI: <https://dx.doi.org/10.54203/scil.2021.wvj64>

**ABSTRACT:** Marine fish, particularly the red snappers, are often exposed to helminth parasitic infestation. As a result of the parasitic infestation, the fish population, the fish weight, and the morphological changes in the fish are shrinking. The present research aimed to find out the intensity and predilection of the helminth ectoparasites over the infection of the red snapper (*Lutjanus argentimaculatus*) and employed the survey method for this purpose. The sampling was carried out by the purposive sampling technique. The sample obtained consisted of 30 fish, 20% of the total red snapper population of 150 fish reared in the floating net cages of Balai Besar Perikanan Budidaya Laut Lampung. The intensity of the fish infestation by a mixture of *Haliotrema epinepheli* and *Benedenia epinepheli* was 132.5 individuals/fish. *Neobenedenia girellae* and *Haliotrema epinepheli* infected fish with an intensity of 149.41 individuals/fish. The 66.7% of *Benedenia epinepheli* had a predilection for the dorsal fin, and 33.3% for the anal fin. In *Neobenedenia girellae*, 57.1% had a predilection for the body surface, 37.2% for the head surface, and 5.7% for the dorsal fin. In conclusion, all sampled fish were positively infected with helminth ectoparasites, including *Neobenedenia girellae*, *Haliotrema epinepheli*, and *Benedenia epinepheli*.

**Keywords:** Ectoparasite, Helminths, Infestation, Red snapper



Prasetya AW, Kismiyati, and Subekti S (2021). Intensity and Predilection of Helminth Parasites of the Red Snapper (*Lutjanus argentimaculatus*). *World Vet. J.*, 11 (3): 499-503.

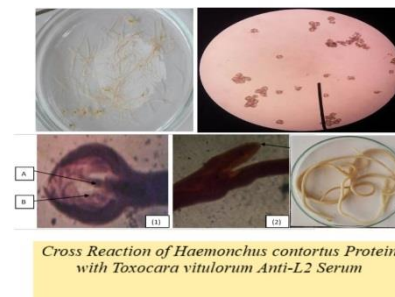
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## Cross Reaction of *Haemonchus contortus* Protein with *Toxocara vitulorum* Anti-L2 Serum Using Western Blot Technique

Asmorowati RW, Kusnoto, and Eliyani H.

*World Vet. J.* 11(2): 504-509, 2021; pii:S232245682100065-11; DOI: <https://dx.doi.org/10.54203/scil.2021.wvj65>

**ABSTRACT:** In the adult stage, *Haemonchus contortus* worms infect the abomasum host causing anemia and even death in animals. However, identifying the *H. contortus* protein can be used as a reference for the diagnosis of diseases. The diagnosis is performed by serological cross-reaction between *H. contortus* protein and anti-L2 *Toxocara vitulorum* (*T. vitulorum*) serum using the western blot technique. The main purpose of the current research was to identify the cross-reaction between *H. contortus* proteins and anti-L2 *T. vitulorum* serum using the western blot technique. *T. vitulorum* worms were collected from the intestine of cattle and *H. contortus* worms were collected from the abomasum of goats. The first step was making antibodies by oral infection of rats with infective eggs (L2) of *T. vitulorum*. The blood was taken 21 days after infection. Then, the blood was centrifuged at 1500 rpm for 10 minutes to get the serum. The second step was making homogenates from the whole worm extract of *H. contortus*. After crushing the worms, it was centrifuged at 5000 rpm for 15 minutes and the supernatant was taken. The supernatant was then analyzed using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) with coomassie brilliant blue staining. The third step was the analysis of *H. contortus* protein with serum anti-L2 *T. vitulorum* using the western blot technique. From the *H. contortus* homogenates analysis using SDS-PAGE, 16 protein bands were obtained. The cross-reactions were 141.3, 81.3, 64. 6, 51.3, 46.8, and 38 kDa. The data from cross-reactions suggested that the *H. contortus* protein cannot be used as a diagnostic material. It is serologically Haemonchosis because it caused false positives with diagnostic Toxocariasis. **Keywords:** Cross reaction, *Haemonchus contortus*, SDS-PAGE, *Toxocara vitulorum*, Western blot



Asmorowati RW, Kusnoto, and Eliyani H (2021). Cross Reaction of *Haemonchus contortus* Protein with *Toxocara vitulorum* Anti-L2 Serum Using Western Blot Technique. *World Vet. J.*, 11 (3): 504-509.

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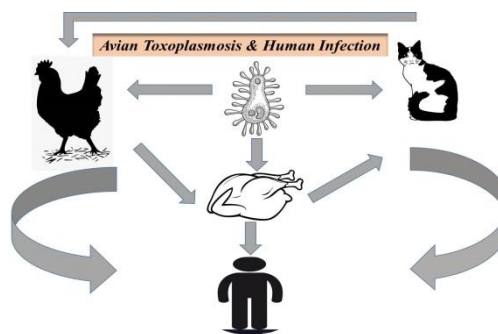
## Review

### A Highlight on Avian Toxoplasmosis: One Health Disease with a Special Reference to the Current Egyptian Situation

Abd El-Ghany WA.

*World Vet. J.* 11(2): 510-520, 2021; pii:S232245682100066-11; DOI: <https://dx.doi.org/10.54203/scil.2021.wvj66>

**ABSTRACT:** This review article was developed to the infection of avian species with *Toxoplasma gondii* (*T. gondii*), diagnosis, pet bird and human infection, and control methods with a special reference to the current status of infection among the Egyptian poultry farms and population. Toxoplasmosis is a zoonotic disease caused by a unicellular, protozoan parasite *T. gondii*. Different domesticated and wild animals, as well as birds can harbor *T. gondii* and may be a potential source of infection to humans. Avian species could be infected with *T. gondii* through the ingestion of contaminated food, soil, and water with oocysts shed in the excreta of infected animals, especially cats. Poor sanitation and hygienic conditions increase the risk of infection. Consumption of food or water, as well as undercooked poultry meat or meat products containing the oocysts of the parasite, are the main sources of human infection with *T. gondii*. Diagnosis of *T. gondii* in the infected host depends on the serological detection of specific antibodies and molecular detection of the parasite. Microscopic demonstration of the oocysts and other developmental stages of the parasite in the intestine, liver, brain, and skeletal muscles tissues is another means for rapid diagnosis. Generally, a high prevalence of the disease is also reported in pet birds. Toxoplasmosis in humans is associated with abortion, congenital disorders, stillbirth, and other complications, especially in immunocompromised patients. Application of hygienic measures, as well as public awareness, are essential for the prevention and control of toxoplasmosis. In different Egyptian governorates, a high prevalence of *T. gondii* has been detected in animals, birds, and humans. High incidence of infection was recorded due to the contact with *Toxoplasma* oocysts shed mainly from infected cats or other carriers. Egyptian chicken and turkey flocks and backyard birds revealed the presence of different developmental stages of the parasite and even its antibodies. In addition, human populations showed signs of toxoplasmosis with severe complications. **Keywords:** Bird, Egypt, Human, *Toxoplasma gondii*, Zoonosis



Abd El-Ghany WA (2021). A Highlight on Avian Toxoplasmosis: One Health Disease with a Special Reference to the Current Egyptian Situation. *World Vet. J.*, 11 (3): 510-520.

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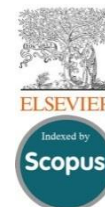
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# Intensity and Predilection of Helminth Parasites of the Red Snapper (*Lutjanus argentimaculatus*)

Agung Wahyu Prasetya<sup>1</sup>, Kismiyati<sup>1\*</sup>, and Sri Subekti<sup>2</sup>

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## ABSTRACT

Marine fish, particularly the red snappers, are often exposed to helminth parasitic infestation. As a result of the parasitic infestation, the fish population, the fish weight, and the morphological changes in the fish are shrinking. The present research aimed to find out the intensity and predilection of the helminth ectoparasites over the infection of the red snapper (*Lutjanus argentimaculatus*) and employed the survey method for this purpose. The sampling was carried out by the purposive sampling technique. The sample obtained consisted of 30 fish, 20% of the total red snapper population of 150 fish reared in the floating net cages of Balai Besar Perikanan Budidaya Laut Lampung. The intensity of the fish infestation by a mixture of *Haliotrema epinepheli* and *Benedenia epinepheli* was 132.5 individuals/fish. *Neobenedenia girellae* and *Haliotrema epinepheli* infected fish with an intensity of 149.41 individuals/fish. The 66.7% of *Benedenia epinepheli* had a predilection for the dorsal fin, and 33.3% for the anal fin. In *Neobenedenia girellae*, 57.1% had a predilection for the body surface, 37.2% for the head surface, and 5.7% for the dorsal fin. In conclusion, all sampled fish were positively infected with helminth ectoparasites, including *Neobenedenia girellae*, *Haliotrema epinepheli*, and *Benedenia epinepheli*.

**Keywords:** Ectoparasite, Helminths, Infestation, Red snapper

## INTRODUCTION

One of the main problems and challenges in red snapper cultivation is disease control (Hussan et al., 2016; Oliveira et al., 2020). Diseases of red snapper could occur in water due to imbalances in the environment, host, and pathogenic organisms (Engering et al., 2013; Hussan et al., 2016; Oliveira et al., 2020). Parasitic infestation and infection might also be attributed to high fish density and inadequate nourishment in fish, as well as poor water and environmental quality (Yadollahi et al., 2013; Hussan et al., 2016).

In nature, red snappers might be susceptible to a number of diseases or parasites (Cable et al., 2017; Oliveira et al., 2020). In the culture environment, the parasites might even be present in far greater numbers and could be lethal to fish (Omeji et al., 2011). Marine fish, particularly the red snappers, are often exposed to parasitic helminth infestation (Montoya-Mendoza et al., 2014; Oliveira et al., 2020). Visual results indicated that the most prevalent ectoparasitic worm in the red snapper was *Benedenia epinepheli*, which was found on the fin and skin (Jithendran et al., 2005; Loh et al., 2020). Meanwhile, *Neobenedenia girellae* (Kishimori et al., 2015), *Hatschekia poche* (Justine et al., 2012), *Haliotrema* species, and *Diplectanum squamatum* N. (Santos et al., 2002) were encountered on the gill.

A parasitic infestation could lead to economic losses. The economic impacts of parasites in fish include reduced fish population for consumption, decreased fish weight, and changes to fish morphology (Iwanowicz, 2011). The prevention and treatment of diseases had thus developed into an important cultivation component. Diseases might be caused by stress, pathogens (e.g., protozoans, bacteria, and viruses), environmental changes (e.g., algae bloom), overdose, and malnutrition (Pikarsky et al., 2004). Different causes could produce distinctive external symptoms of illness in fish, including sudden death, changes in behavior, loss of appetite, and bleeding in places where scales have peeled off (Madhun et al., 2015). Parasites infest or infect the red snapper in a number of ways by, for instance, parasitic contamination of the water in which red snappers are cultivated, direct contact, or rubbing with red snappers contaminated with parasites. In this case, the spread of the sick red snappers would have negative effects, especially if the density is too high. Furthermore, the equipment previously used to hand or transport parasite-contaminated red snappers would also cause parasite infestation or infection (Melianawati and Aryati, 2012).

Helminths are classified according to the International code. Every parasite belongs to particular phylum, class, order, genus, and species. Helminths per se are assigned to four different phyla, namely Platyhelminthes, Nematelminthes, Acanthocephala, and Annelida (Gibson et al., 2014; Morand et al., 2015).

SHORT COMMUNICATION  
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The phylum Platyhelminthes is characterized by a dorsoventral flattening of the body and bilateral symmetry, whereby its organs are embedded within the parenchyma tissues, and there are no respiratory and circulatory systems. The phylum Platyhelminthes is composed of two classes, namely Trematode and Cestode (Hahn et al., 2014; Morand et al., 2015). The parasites of the Trematode class have an ovoid, leaf-like, unsegmented body, and an incomplete digestive tract (a sac with one or two suckers for attachment). Worms in this class have a hermaphrodite reproductive system. This class is divided into three subclasses of Monogenea, Aspidogastrea, and Digenea (Crotti, 2013). Parasites of the order Dactylogyridae of the class Trematode are parasites of aquatic vertebrates, for example, the Reptiles (crocodiles), Amphibians (frogs), and Pisces (fishes). The majority of the parasites in the order Dactylogyridae are ectoparasites with a direct life cycle. The order Dactylogyridae of the class Trematoda has a distinctive feature on its posterior part of the body, that is, a haptor with a pair of hooks to attach itself to its host (Gibson et al., 2014).

There are a number of ectoparasitic worm types that infect the red snapper, namely *Benedenia*, *Neobenedenia*, and *Diplectanum*. These three worms are described as follows. *Benedenia* is a colorless ectoparasite that attacks the surface of the body. In particular, *Benedenia* causes fish to lose their appetite and have abnormalities in the way they swim on the bottom or surface of the container or floating net cage. Severe infection with *Benedenia* could cause wounds or ulcers (a pus-like liquid) on the skin and eventually secondary bacterial and fungal infection (Zhang et al., 2017). *Neobenedeniagirellae* shows a higher degree of pathogenicity than *Benedeniaepinephelibe* because *Neobenedeniagirellae* infects not only the skin but also the eye and causes blindness. Severely infected fish lose their appetite, have dark body colors, and have irregular swimming patterns and movements (Zhang et al., 2017). *Neobenedeniamelleni* shows clinical symptoms in the skin, such as hyperemia and bleeding, and often infects the eye, causing damages to the cornea and blindness of the fish (Trujillo-González et al., 2015). *Diplectanum* disrupts the respiratory function in fish. The resulting wounds would lead to secondary bacterial infection. The growth of fish might be delayed, and the body weight and appetite are reduced. The fish infected by *Diplectanum* had pale gills, which produced excessive amounts of mucous, and a pale body (Cecchini et al., 1998). These three worms (*Benedenia*, *Neobenedenia*, and *Diplectanum*) are responsible for the diseases that the red snappers suffer from. The present research was then conducted to figure out the intensity and predilection of the helminth ectoparasites of the red snappers.

## MATERIALS AND METHODS

### Ethical approval

The present research employed the survey method. The sample of red snappers (*L. argentimaculatus*) was obtained by the purposive sampling method according to specified criteria. The sample had sizes specified by the researchers from the location of the floating net cages for one week. In this case, the red snappers were found to be 25–35 centimeters long.

The red snappers enrolled as samples were taken from one of seven floating net cages at Balai Besar Perikanan Budidaya Laut (BBPBL) Lampung with the number 30, or accounting for 20% of the total population of 150 red snappers, which were reared in  $1 \times 1 \times 1.5 \text{ m}^3$  polyethylene nets. The extracted sample was transported to the Laboratory of Fish Health and Environment of BBPBL Lampung, Indonesia, for helminth parasites examination.

The current research used a fishing net, a plastic bucket for fish sampling, and a Kemmerer bottle for water sampling. The instruments used to calculate the intensity and predilection of the helminth parasites were a set of surgical instruments (scalpel, surgical scissors, forceps, object-glass, and cover glass), pipette, towel paper, ruler, digital scale, digital camera, label paper, microtube, and trinocular microscope. Instruments used to measure water quality included a pH meter for pH measurement, a refractometer for measuring salinity, a Dissolved Oxygen (DO) meter for measuring dissolved oxygen, and a thermometer for measuring temperature.

The main materials used for the present research were the red snappers (*L. argentimaculatus*) and the seawater sample from the floating net cage location. Meanwhile, 250 mL of 5% glycerin was used to study the helminth parasite. The current research was undertaken from February through March 2016 in the Laboratory of Fish Health and Environment, BBPBL Lampung, and the Education Laboratory of the Faculty of Fisheries and Marine Affairs, Universitas Airlangga, while the sampling was performed at the location of the floating net cages of BBPBL Lampung.

## RESULTS

The results of the present research concerned the intensity of the helminth ectoparasites, the predilection of helminth ectoparasites, the distribution of predilection of helminth ectoparasites, and the water quality parameters.

### Intensity of helminth ectoparasites

From the investigation of the 30 samples of red snappers (*Lutjanus argentimaculatus*) that were cultivated in a floating net cage of BBPBL Lampung, helminth ectoparasites were found. The results of the sample examination indicated that a single sample fish could be infested with more than one type of helminth ectoparasite. Of the 30 sample

fish examined, 12 were only infested by *Haliotrema epinepheli* (sole infestation), including 1534 worms found with an intensity of 127.83 individuals/fish. Aside from those fish, 6 were infected with a mixture of *Benedenia epinepheli* and *Haliotrema epinepheli*, with 795 worms found with an intensity of 132.5 individuals/fish, and 12 were infested with a mixture of *Neobenedenia girellae* and *Haliotrema epinepheli*, having 1793 worms with an intensity of 149.41 individuals/fish.

### **Predilection of helminth ectoparasites**

Observations on the predilection of the helminth ectoparasites were performed in the Laboratory of Fish Health and Environment of BBPBL Lampung. The observations were carried out on macroscopic and microscopic bases. The predilection test procedure was performed by scraping off all body parts of the fish, followed by observations under a microscope at 100 × and 400 × magnifications. Predilection sites for the helminth ectoparasites were the body surface of the fish and the fins, including the dorsal, pectoral, anal, and pelvic fins. The predilection study on the gills was conducted by observing the histopathological preparations of the fins infested by the ectoparasitic worms. The examination included changes in the structure and appearance of the organs infested by the ectoparasitic worms.

According to the examination results of the worms in the red snappers, *Benedenia epinepheli* had a round, elongated body with a length of 2.06 to 3.05 mm, a width of 0.67 to 1.51 mm, and a diameter of 0.50 to 0.78 mm. *Benedenia epinepheli* had attachment organs 0.16-0.25 mm long and 0.12 to 0.27 mm wide, a pharynx of 0.16-0.35 mm long and 0.18-0.25 mm wide, accessory sclerites of 0.07 to 0.11 mm long, anterior hamuli of 0.08 to 1.21 mm long, and posterior hamuli of 0.04 to 0.07 mm long.

*Neobenedenia girellae* was found to have a round elongated body with a length of 4.6 to 5.7 mm, and a width of 1.5 to 2.5 mm. In addition to an opisthaptor of 1.2-1.5 mm in size, *Neobenedenia girellae* also had a pair of attachment organs with the length of 0.14 to 0.22 mm and width of 0.17 to 0.29 mm, a pharynx with a length from 0.51 to 0.71 and a width of 0.39 to 0.42 as well as accessory sclerites with a length of 0.25 to 0.31 mm, anterior hamuli of 0.28-0.39 mm long, and posterior hamuli of 0.11-0.18 mm long. Meanwhile, *Haliotrema epinepheli* was found to be 200.8 µm in length and 48.12 µm in width, and also had a pharynx 15.40 to 18.71 µm in diameter.

### **Distribution of the predilection of helminth parasites**

66.7% of the *Benedenia epinepheli* that infest the red snappers preferred the dorsal fin, and 33.3% preferred the anal fin. No *Benedenia epinepheli* infestation was found on the caudal, pectoral, and ventral fins, and head. For the *Neobenedenia girellae* infestation in the red snappers, a majority of 57.1% had a high predilection for the body surface, 37.2% a medium predilection for the head, and 5.7% a low predilection for the dorsal fin. No predilection was indicated for the caudal, pectoral, anal, and ventral fins. Meanwhile, a 100% predilection for *Haliotrema epinepheli* was indicated for the gills and none for other parts of the body. As for the histopathologic observations of the gills, the results indicated that *Haliotrema epinepheli* had a predilection for the lamellae part of the red snapper gills.

### **Water quality parameters**

The measurement of the water quality in terms of pH, DO, and salinity was performed once a week in the morning. The results of the observation of some indicators of water quality in the floating net cages of BBPBL Lampung in 2016 indicated that the water was in a normal condition for the life of the cultivated fish. The data indicated that the values were within normal ranges, where the temperature was between 30°C and 31°C, salinity was reported as 5 ppt, pH was estimated at 7, DO was between 6 and 8.37 mg/L, and the ammonia was also between 0.044 and 0.221 mg/L.

## **DISCUSSION**

Based on the results of the examination of the body surface and the gills of red snappers (*Lutjanus argentimaculatus*), it was found that three parasitic worm species infest the red snappers in the floating net cages of BBPBL Lampung, namely *Benedenia epinepheli*, *Neobenedenia girellae*, and *Haliotrema epinepheli*. The helminth parasites *Benedenia epinepheli* and *Neobenedenia girellae* were encountered on the body surface of the red snappers, while *Haliotrema epinepheli* were found in the gills. The present findings are similar to Kritsky's (2012) review in the Persian Gulf, the eastern, Indo-west Pacific Ocean, and Mexico gulfs snappers.

The helminth ectoparasite *Benedenia epinepheli*, which infested the body surface of the red snappers as stated previously by Ravi and Yahaya (2016), had a predilection, especially on the dorsal and anal fins and in the skin area. When examining the ectoparasitic worms in the red snappers, *Benedenia epinepheli* was found to infest the dorsal fin area of 4 out of 30 sample fish, and infestation on the anal fin was rare.

The present research also discovered an infestation of the ectoparasitic worm *Neobenedenia girellae*. During the investigation, *Neobenedenia girellae* was found on the body surface, especially in the head area, in 13 out of 30 sample red snappers. It can be said that the worms had the highest prevalence on the body surface and rarely showed up on the

dorsal fin. *Neobenedenia girellae* had a predilection for the skin around the head and would also cause blindness, but was not found on the dorsal fin and the remaining body parts such as the gills and the internal organs.

The ectoparasitic worm *Haliotrema epinepheli* was also encountered in the present research. The predilection of the worm was exclusively for the gills. All sample fish infested by *Haliotrema epinepheli* demonstrated clinical symptoms of excessive mucus and swimming on the water surface. A severe infection of *Haliotrema epinepheli* could damage the filaments of the gills and cause the infected fish to suffer respiratory disorders or, in the worst case, die.

Based on the visual results, all sampled fish were infested by *Haliotrema epinepheli* in the gills. The *Benedenia epinepheli* infestation was present on the dorsal and anal fins, while the *Neobenedenia girellae* infestation occurred on the body surface, head, and dorsal fin, suggesting that *Haliotrema epinepheli* predominantly infested fish gills, eliminating competition between worm species in the areas. The fish infested by the helminth ectoparasite *Haliotrema epinepheli* rarely suffered wounds in their gill lamellae.

Although many of the red snappers (*Lutjanus argentimaculatus*) cultivated in the floating net cages at BBPBL Lampung were infested by *Haliotrema epinepheli* in the gills, none of the fish died. *Benedenia epinepheli* and *Neobenedenia girellae* did not leave any serious impact on the red snappers' health in small numbers. However, if *Benedenia epinepheli* and *Neobenedenia girellae* were present in significant numbers, they could be fatal to the fish (Melianawati and Aryati, 2012).

Based on the results of the examination on the 30 sample red snappers, 100% of the fish were positively infested with helminth ectoparasites, suggesting that the infestation intensity in floating net cages was very high. The infestation with helminth ectoparasites in the cultivation of floating net cages occurred due to the poor fish rearing and uncontrolled water quality since the cultivation was weather-dependent. Another factor presumed to decrease the red snappers' physical endurance was the stress resulted from the flow of water and net cages spoilage from the attachment of parasites and other microorganisms which blocked water circulation, which was mentioned by Cooper (2015). In addition, stress could also be caused by high density, which allowed parasites to develop rapidly, with high density triggered competition for space, food, and oxygen. The mortality rate from infestation with helminth ectoparasites at this cultivation location (East Java) was categorized as so high that the red snapper population in these floating net cages was smaller compared to other populations.

## CONCLUSION

All of the fish samples were positively infected by helminth ectoparasites, including *Neobenedenia girellae*, *Haliotrema epinepheli*, and *Benedenia epinepheli*. Therefore, in the East Java region, Ocean fish are most likely to be infected with these parasites. In fish inspections, parasite-related infections should be considered cautiously, especially in marine fish like Red Snapper (*Lutjanus argentimaculatus*).

## DECLARATIONS

### Authors' contribution

All the authors contributed equally to this study.

### Competing interests

The authors have not declared any conflict of interest.

### Ethical considerations

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by the authors.

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