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B. A. Lubisi - Virology, MED Programme, ARC - Onderstepoort Veterinary Institute, No. 100 Old Soutpan Road, Onderstepoort, Tshwane, 0110, South Africa
Google Scholar profile: <https://scholar.google.com/citations?user=Wwcc5-8AAAAJ&hl=en>
Interest area: Virology

Girija Regmi - Department of Cardiovascular Biology, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma, USA
<https://orcid.org/0000-0001-6827-3783>
Google Scholar profile: <https://scholar.google.com/citations?user=JRhK5-sAAAAJ&hl=en>
Interest area: Anatomy - Animal Hygiene, Husbandry, Nutrition, and Food Control - Animal Nutrition - Animal Reproduction - Animal Science - Antimicrobial resistance - Bacteriology - Biological Sciences - Biomedical Sciences - Hematology - Immunohistochemistry - Microbiology - Molecular Biology - Veterinary Anatomy, Histology, and Physiology - Veterinary Medicine - Veterinary Medicine and Infectious Diseases - Veterinary Pathology - Veterinary Science - Zoonoses

Widya Paramita Lokapirnasari - Professor, Department of Animal Husbandry, Airlangga University, FKH, Kampus C Unair, Jl Mulyorejo, Surabaya, Indonesia
<https://orcid.org/0000-0002-0319-7211>
Google Scholar profile: <https://scholar.google.co.id/citations?user=eS3yVQAAAAJ&hl=id>
Interest area: Animal Nutrition - Cattle Husbandry - Feed Supplements - Polymerase Chain Reaction - Poultry Husbandry - Probiotics

Ayman Abdel-Aziz Swelum - Professor of Theriogenology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt; Department of Animal Production, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia
<http://orcid.org/0000-0003-3247-5898>
Google Scholar profile: <https://scholar.google.com/citations?user=OZTI3poAAAAJ&hl=en>
Profile: <http://www.staffdata.zu.edu.eg/en/ShowData/18313> <https://faculty.ksu.edu.sa/ar/aswelum>
Interest area: Animal Reproduction - Animal Production - Embryo transfer - Artificial Insemination

Mario Manuel Dinis Ginja Department of Veterinary Sciences, Center for Research and Agro-Environmental and Biological Technologies, University of Trás-os-Montes and Alto Douro, Portugal
<https://orcid.org/0000-0002-0464-7771>
Publons profile: <https://publons.com/researcher/1180094/mario-manuel-dinis-ginja/>
Interest area: Orthopaedics - Radiology (Diagnostic) - Sonography - Veterinary Medicine - Veterinary Science

Panagiotis E Simitzis - Laboratory of Animal Breeding and Husbandry, Department of Animal Science, Agricultural University of Athens, 75 Iera Odos, 11855, Athens, Greece
<http://orcid.org/0000-0002-1450-4037>
Google Scholar profile: <https://scholar.google.com/citations?user=14F6cAQAAAAJ&hl=en>
Interest area: Dietary Antioxidants - Feed Supplements - Animal Behaviour - Animal Welfare - Livestock Management - Poultry Husbandry - Sheep Husbandry - Swine Husbandry - Products' Quality Assessment

Gul Ahmad - Associate Professor of Biology (Tenured), Department of Natural Sciences, School of Arts & Sciences, Peru State College, Peru, Nebraska 68321, USA
Google Scholar profile: <https://scholar.google.com/citations?user=W0IDNKUAAAAJ&hl=en>

Bartosz Kieronczyk - Poznan University of Life Sciences, Poznan, Greater Poland, Poland
<https://orcid.org/0000-0001-6006-117X>
Google Scholar profile: <https://scholar.google.pl/citations?user=SyprUmAAAAJ&hl=en>
Interest area: Animal Nutrition - Animal Science - Antimicrobial resistance - Aquaculture - Feed Supplements - Livestock Management - Livestock Products Technology - Microbiology - Physiology - Poultry Science - Waste Management of Agro Products

Alberto Elmi - University of Bologna, Ozzano dell'Emilia, Bologna, Italy
<https://orcid.org/0000-0002-7827-5034>
Google Scholar profile: <https://scholar.google.it/citations?user=ej4LzNgAAAAJ&hl=it>
Interest area: Animal Reproduction - Laboratory Animal Research - Laboratory Medicine - Physiology - Swine Medicine - Wildlife

Editorial board

Suresh H. Basagoudanavar - FMD Vaccine Research Laboratory, Indian Veterinary Research Institute, Bangalore- 560024, Karnataka, India
<https://orcid.org/0000-0001-7714-3120>
ResearchGate profile: <https://www.researchgate.net/profile/Suresh-Basagoudanavar>
Interest area: Biotechnology - Immunology - Virology

Gyanendra Gongal - Senior Public Health Officer (Food safety, zoonoses and One Health), World Health Emergency Programme, WHO Regional Office for south East Asia, New Delhi, India
<https://orcid.org/0000-0002-6539-7569> Google Scholar profile: <https://scholar.google.com/citations?user=XNCypDcAAAAJ&hl=en>
Interest area: Public Health - Zoonoses - One Health

Md. Tanvir Rahman - Department of Microbiology and Hygiene, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
<https://orcid.org/0000-0001-5432-480X>
Google Scholar profile: <https://scholar.google.com/citations?user=vp6xgh0AAAAJ&hl=en>
Interest area: Antimicrobial resistance - Virulence-Food hygiene- Public Health - Vaccine - One Health

Fouad Kasim Mohammad - Professor Emeritus, Pharmacology & Toxicology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq
Google Scholar profile: <https://scholar.google.com/citations?user=zgCIA4UAAAAJ&hl=en>
Interest area: Pharmacology - Toxicology

Joao Simoes - Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal
<https://orcid.org/0000-0002-4997-3933>
Google Scholar profile: <https://scholar.google.com/citations?user=ftLFW-sAAAAJ&hl=en>
Interest area: Large Animal Medicine - Mastitis - Reproductive medicine - Veterinary Medicine

Abdelaziz ED-DRA - Department of Biology, Faculty of Science, Moulay Ismail University, BP. 11201 Zitoune, Meknes, Morocco
<https://orcid.org/0000-0003-3273-1767>
Google Scholar profile: <https://scholar.google.com/citations?user=ftL-1V0AAAAJ&hl=en>
Interest area: Antimicrobial resistance - Clinical Microbiology - Food - Food/Meat Hygiene - Polymerase Chain Reaction

Filippo Giarratana - Department of Veterinary Medicine, University of Messina, Polo Universitario dell'Annunziata, 98168 Messina, Italy
<https://orcid.org/0000-0003-0892-4884>
Google Scholar profile: <https://scholar.google.com/citations?user=lut-WbIAAAAAJ&hl=it>
Interest area: Antimicrobial resistance - Bacteriology - Food/Meat Hygiene - Plant Science - Essential oils

Eduardo Jorge Boeri - Institute of Zoonosis Luis Pasteur, Buenos Aires, Argentina
<https://orcid.org/0000-0001-8535-0306>
Google Scholar profile: https://scholar.google.com/citations?user=aerl_4oAAAAJ&hl=en&oi=sra
Interest area: Brucellosis - Microbiology - Veterinary Medicine - Veterinary Public Health - Zoonoses

Kumar Venkitanarayanan - Graduate Programs Chair, Honors and Pre-Vet Programs Advisor, Department of Animal Science, University of Connecticut, Storrs, CT 06269, USA
Google Scholar profile: <https://scholar.google.com/citations?hl=en&user=N9CY28AAAAJ>
Interest area: Bacteriology - Clinical Microbiology - Infectious Diseases - Veterinary Medicine

Karim El-Sabrouh - Poultry Production Department, Alexandria University, Alexandria, Egypt
<https://orcid.org/0000-0003-2762-2363>
Google Scholar profile: <https://scholar.google.com/citations?hl=en&user=q-1JH8AAAAJ>
Interest area: Poultry Husbandry

Ali Aygun - Selçuk University, Agriculture Faculty, Department of Animal Science, Konya, TURKEY
<https://orcid.org/0000-0002-0546-3034>
Google Scholar profile: <https://scholar.google.com/citations?hl=en&user=nZsp5IAAAAAJ>
Interest area: Poultry Husbandry - Poultry Medicine

Ionel D. Bondoc - Associate Professor, Department of Public Health, Faculty of Veterinary Medicine Iasi, University of Life Sciences "Ion Ionescu de la Brad" Iasi, Romania
<https://orcid.org/0000-0002-5958-7649>
Google Scholar profile: <https://scholar.google.ro/citations?user=-dUf6oYAAAAJ&hl=ro>
Publons Profile: <https://publons.com/researcher/741287/ionel-bondoc/>
Interest area: Dairy Science - Epidemiology - Food Science - Food Technology - Food Law - One Health - Parasitology - Meat Inspection - Pathogens - Foodborne Diseases - Food Toxicology - Veterinary Public Health - Wildlife Diseases - Zoonoses

Liliana Agullar-Marcelino - National Center for Disciplinary Research in Animal Health and Safety, National Institute for Agricultural and Livestock Forestry Research, Mexico
<https://orcid.org/0000-0002-8944-5430>
 Google Scholar profile: <https://scholar.google.ro/citations?hl=ro&user=ZbMMP-UAAAAJ>
 Interest area: Biology - Ethnoveterinary - Parasitology - Veterinary Medicine - Veterinary Public Health

Anut Chantiratikul - Department of Agricultural Technology, Faculty of Technology, Maharakham University, Muang, Maharakham Province 44150 Thailand
<https://orcid.org/0000-0002-8313-5802>
 Google Scholar profile: <https://scholar.google.ro/citations?hl=ro&user=QogjWpgAAAAJ>
 Interest area: Biology - Animal Nutrition

Nuh Kilic - Department of Surgery, Faculty of Veterinary Medicine, Adnan Menderes University, Turkey
<https://orcid.org/0000-0001-8452-161X>
 Google Scholar profile: <https://scholar.google.ro/citations?hl=ro&user=APVrx1cAAAAJ>
 Interest area: Large Animal Medicine - Surgery - Veterinary Medicine

Hanna Markiewicz - Milk Examination Laboratory, Kazimierz Wielki University in Bydgoszcz, Poland
<https://orcid.org/0000-0001-8225-0481>
 ResearchGate profile: <https://www.researchgate.net/scientific-contributions/H-Markiewicz-10381112>
 Interest area: Large Animal Medicine - Mastitis

N. De Briyne - Federation of Veterinarians of Europe, Brussels, Belgium
<https://orcid.org/0000-0002-2348-930X>
 Google Scholar profile: <https://scholar.google.ro/citations?hl=ro&user=BOhORAAAAAJ>
 Interest area: Animal Science - Antimicrobial resistance

Hasan Meydan - Akdeniz University, Faculty of Agriculture, Antalya, Turkey
<https://orcid.org/0000-0003-4681-2525>
 Google Scholar profile: <https://scholar.google.ro/citations?hl=ro&user=T2uHga0AAAAJ>
 Interest area: Biotechnology - Genetics - Veterinary Medicine

Suleyman Cilek - Kirikkale Universitesi, Kirikkale, kirikkale, Turkey
<https://orcid.org/0000-0002-2352-649X>
 ResearchGate profile: <https://www.researchgate.net/scientific-contributions/Suleyman-Cilek-2092525513>
 Interest area: Animal Nutrition - Animal Nutrition - Animal Reproduction - Animal Reproduction - Animal Reproduction - Breeding - Cattle Husbandry - Cattle/buffalo management - Equine Medicine - Genetics - Livestock Management - Mastitis - Molecular Genetics - Poultry Husbandry - Poultry Husbandry - Sheep Husbandry - Sheep Husbandry - Small Animal Medicine - Swine Husbandry - Veterinary Medicine

Rodrigo Alberto Jerez Ebensperger - University of Zaragoza, Spain
 Interest area: Animal Reproduction - Artificial Insemination - Biotechnology - Breeding - Embryo Transfer Technology - Equine Medicine - Large Animal Medicine - Livestock Management - Small Animal Medicine - Veterinary Medicine - Wildlife

Parag Nigam - Department of Wildlife Health Management, Wildlife Institute of India, Dehradun, India
 ResearchGate profile: <https://www.researchgate.net/profile/Parag-Nigam>
 Interest area: Veterinary Medicine - Veterinary Public Health - Wildlife - Zoonoses

Alessandra Pelagalli - Department of Advanced Biomedical Sciences, University of Naples Federico II, Italy
<https://orcid.org/0000-0002-1133-4300>
 Google Scholar profile: <https://scholar.google.ro/citations?hl=ro&user=T1IZqmAAAAJ>
 Interest area: Physiology

Jamal Gharekhani - Senior researcher, Iranian Veterinary Organization (IVO), Hamedan, Iran
<https://orcid.org/0000-0001-5882-8861>
 Google Scholar profile: <https://scholar.google.ro/citations?hl=ro&user=vlhjoBEAAAAJ>
 Interest area: Parasitology - Pathobiology - Veterinary Public Health

Ipsita Mohanty - Postdoctoral Research Fellow, Children's Hospital of Philadelphia Research Institute, (CHOP), Philadelphia
<https://orcid.org/0000-0003-0894-4770>
 Google Scholar profile: <https://scholar.google.ro/citations?hl=ro&user=anWIO7IAAAAAJ>
 Interest area: Pharmacology - Toxicology - Physiology - Cardiology

Alejandro Hidalgo - Preclinical Science Department, Faculty of Medicine, Universidad de La Frontera, Temuco, Chile
<https://orcid.org/0000-0002-2247-4878>
 Google Scholar profile: <https://scholar.google.ro/citations?hl=ro&user=5veJgSAAAAAJ>
 Interest area: Zoonotic parasitic diseases - Parasite phylogeny - Zoology - Parasitology

Hua-Ji Qiu - Professor, Harbin Veterinary Research Institute (HVRI), Chinese Academy of Agricultural Sciences (CAAS), Harbin, Heilongjiang, 150069, P.R. China
<https://orcid.org/0000-0003-4880-5687>
 Profile: http://www.hvri.ac.cn/zjig/ctxd/zkzrbctxd/sx_20180726100149743651/index.htm
 Interest area: Classical swine fever - African swine fever - Pseudorabies - Innate and adaptive immunity - Virus-host interactions - Pathogenesis - Epidemiology - Vaccines - Diagnostic assays - Probiotics

Hasria Alang - Biology Lecturer at STKIP-PI Makassar, Makassar, Indonesia
<https://orcid.org/0000-0001-9393-9575>
 Google Scholar profile: <https://scholar.google.ro/citations?hl=ro&user=NpwjancAAAAJ>
 Interest area: Microbiology - Molecular Biology

Belgin Siriken - Professor, Department of Water Products Diseases, Faculty of Veterinary Medicine, Ondokuz Mayıs University, Kurupelit Campus, 55200 Samsun, Turkey
<https://orcid.org/0000-0002-5793-1792>
 Google Scholar profile: <https://scholar.google.ro/citations?hl=ro&user=jpuWvaUAAAAJ>
 Interest area: Food - Food science - Food Technology - Food borne diseases - Antibiotic resistance - One Health - Veterinary Public Health

Hussein Awad Hussein - Professor of Internal Veterinary Medicine, Department of Animal Medicine, Faculty of Veterinary Medicine, Assiut University, Assiut 71526, Egypt
<https://orcid.org/0000-0003-0449-8283>
 Google Scholar profile: <https://scholar.google.ro/citations?hl=ro&user=olySPI8AAAAJ>
 Interest area: Internal Medicine - Spectrophotometry - Ultrasonography - Parasitological analysis - Blood gas analysis - Metabolic profiling - Veterinary Medicine - Large Animal Medicine - Equine Medicine - Mastitis

Tanko Polycarp Nwunuji - Senior lecturer, Department of Veterinary Microbiology and Pathology, Faculty of Veterinary Medicine, University of Jos, Plateau State, Nigeria
<https://orcid.org/0000-0003-1459-2564>
 Google Scholar profile: <https://scholar.google.ro/citations?hl=ro&user=MD7ehVwAAAAJ>
 Interest area: Clinical and Anatomic Pathology - Oncology - Fisheries with special interest in bacterial diseases of fishes and other diseases associated with aquaculture management - Diseases of small and large ruminants - Laboratory animal medicine - Diseases of Dogs, horses and pigs as well as non-infectious diseases such as Diabetes and stress-induced pathologies

Md. Ahaduzzaman - Associate Professor, Department of Medicine and Surgery, Faculty of Veterinary Medicine, Chittagong Veterinary and Animal Sciences University, Bangladesh
<https://orcid.org/0000-0002-0568-0506>
 Google Scholar profile: https://scholar.google.ro/citations?hl=ro&user=u6x_8FKAAAAJ
 Interest area: Antimicrobial resistance - Infectious Diseases - Poultry Medicine - Veterinary Microbiology and Parasitology - Veterinary Public Health - Veterinary Science - Meta-analysis - Phylogenetic analysis

Vanessa S. Cruz - Professor, Department of Veterinary Medicine, Catholic University Center of East Minas (Unileste), Avenue President Tancredo de Almeida Neves, 3500, University District, Coronel Fabriciano - MG, Brazil
<https://orcid.org/0000-0002-8914-5964>
 Profile: <http://lattes.cnpq.br/8788967925940484>
 Interest area: Cancer - Molecular Biology - Veterinary Medicine - Veterinary Pathology - Small Animal Clinic and Surgery (oncology, geriatrics, breeding and behavior of dogs and cats)

R.Umaya Suganthi - Principal Scientist, ICAR-National Institute of Animal Nutrition and Physiology (ICAR-NIANP), Government of India, Bangalore 560 030, Karnataka, India
<https://orcid.org/0000-0002-7710-6271>
 Google Scholar Profile: <https://scholar.google.co.in/citations?user=6VEZ7XMAAAAAJ&hl=en>
 Interest area: Antimicrobial resistance - Antibiotic growth promoters in poultry and their alternatives - Phytochemicals - Oxidative stress and antioxidants - Mycotoxin toxicity and amelioration - Selenium and selenoproteins

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Editorial Office

Veterinary World Star, Gulshan Park, NH-8A, Chandrapur Road,
 Wankaner - 363621, Dist. Morbi (Gujarat), India
 E-mail: editorveterinaryworld@gmail.com
 Website: www.veterinaryworld.org

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Dr. Anjum V. Sherasiya

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E-mail: editorveterinaryworld@gmail.com

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Research (Published online: 04-05-2020)

1. Effects of body condition score and estrus phase on blood metabolites and steroid hormones in Saanen goats in the tropics

Pradita Iustitia Sitaresmi, Budi Prasetyo Widyobroto, Sigit Bintara and Diah Tri Widayati
Veterinary World, 13(5): 833-839

[Abstract \(http://www.veterinaryworld.org/Vol.13/May-2020/1.html\)](http://www.veterinaryworld.org/Vol.13/May-2020/1.html)

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Research (Published online: 05-05-2020)

2. Individual variation in fresh and frozen semen of Bali bulls (*Bos sondaicus*)

R. Indriastuti, M. F. Ulum, R. I. Arifiantini and B. Purwantara
Veterinary World, 13(5): 840-846

[Abstract \(http://www.veterinaryworld.org/Vol.13/May-2020/2.html\)](http://www.veterinaryworld.org/Vol.13/May-2020/2.html)

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Review (Published online: 06-05-2020)

3. The different hormonal system during exercise stress coping in horses

Adriana Ferlazzo, Cristina Cravana, Esterina Fazio and Pietro Medica
Veterinary World, 13(5): 847-859

[Abstract \(http://www.veterinaryworld.org/Vol.13/May-2020/3.html\)](http://www.veterinaryworld.org/Vol.13/May-2020/3.html)

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Research (Published online: 08-05-2020)

4. Ophthalmic findings in sheep treated with closantel in Curitiba, Brazil

Marianna Bacellar-Galdino, Fabiano Montiani-Ferreira, Andre Tavares Somma, Ricardo Guilherme D'Otaviano de Castro Vilani and Ivan Roque de Barros Filho
Veterinary World, 13(5): 860-864

[Abstract \(http://www.veterinaryworld.org/Vol.13/May-2020/4.html\)](http://www.veterinaryworld.org/Vol.13/May-2020/4.html)

[PDF \(http://www.veterinaryworld.org/Vol.13/May-2020/4.pdf\)](http://www.veterinaryworld.org/Vol.13/May-2020/4.pdf)

Research (Published online: 09-05-2020)

5. Celery (*Apium graveolens*) as a potential antibacterial agent and its effect on cytokeatin-17 and other healing promoters in skin wounds infected with methicillin-resistant *Staphylococcus aureus*

Yos Adi Prakoso, Chylen Setiyo Rini, Asih Rahayu, Miarsono Sigit and Dyah Widhawati
Veterinary World, 13(5): 865-871

[Abstract \(http://www.veterinaryworld.org/Vol.13/May-2020/5.html\)](http://www.veterinaryworld.org/Vol.13/May-2020/5.html)

[PDF \(http://www.veterinaryworld.org/Vol.13/May-2020/5.pdf\)](http://www.veterinaryworld.org/Vol.13/May-2020/5.pdf)

Research (Published online: 09-05-2020)

6. Accuracy of methods for diagnosing heart diseases in cats

Tanarut Laudhittirut, Natrada Rujjivipat, Kornnicha Saringkarisate, Peeraya Soponpattana, Teerawat Tunwichai and Sirilak Disatian Surachetpong
Veterinary World, 13(5): 872-878

[Abstract \(http://www.veterinaryworld.org/Vol.13/May-2020/6.html\)](http://www.veterinaryworld.org/Vol.13/May-2020/6.html)

[PDF \(http://www.veterinaryworld.org/Vol.13/May-2020/6.pdf\)](http://www.veterinaryworld.org/Vol.13/May-2020/6.pdf)

Research (Published online: 11-05-2020)

7. Profile of follicle-stimulating hormone and polymorphism of follicle-stimulating hormone receptor in Madrasin cattle with ovarian hypofunction

Budi Utomo, Emmanuel Djoko Putranto and Amaq Fadholly
Veterinary World, 13(5): 879-883

[Abstract \(http://www.veterinaryworld.org/Vol.13/May-2020/7.html\)](http://www.veterinaryworld.org/Vol.13/May-2020/7.html)

[PDF \(http://www.veterinaryworld.org/Vol.13/May-2020/7.pdf\)](http://www.veterinaryworld.org/Vol.13/May-2020/7.pdf)

Research (Published online: 14-05-2020)

8. Single nucleotide polymorphisms in the growth hormone receptor gene and *Alu1* polymorphisms in the diacylglycerol acyltransferase 1 gene as related to meat production in sheep

Nada H. Altwaty, Lamiaa M. Salem and Karima F. Mahrous
Veterinary World, 13(5): 884-889

[Abstract \(http://www.veterinaryworld.org/Vol.13/May-2020/8.html\)](http://www.veterinaryworld.org/Vol.13/May-2020/8.html)

[PDF \(http://www.veterinaryworld.org/Vol.13/May-2020/8.pdf\)](http://www.veterinaryworld.org/Vol.13/May-2020/8.pdf)

Research (Published online: 14-05-2020)

9. Cardiac troponin I as a cardiac biomarker has prognostic and predictive value for poor survival in Egyptian buffalo calves with foot-and-mouth disease

Mahmoud Aly, Mohamed Nayel, Akram Salama, Emad Ghazy and Ibrahim Elshahawy
Veterinary World, 13(5): 890-895

[Abstract \(http://www.veterinaryworld.org/Vol.13/May-2020/9.html\)](http://www.veterinaryworld.org/Vol.13/May-2020/9.html)

[PDF \(http://www.veterinaryworld.org/Vol.13/May-2020/9.pdf\)](http://www.veterinaryworld.org/Vol.13/May-2020/9.pdf)

Research (Published online: 15-05-2020)

10. Prevalence of virulence factor, antibiotic resistance, and serotype genes of *Pasteurella multocida* strains isolated from pigs in Vietnam

Hung Vu-Khac, T. T. Hang Trinh, T. T. Giang Nguyen, X. Truong Nguyen and Thi Thanh Nguyen
Veterinary World, 13(5): 896-904

[Abstract \(http://www.veterinaryworld.org/Vol.13/May-2020/10.html\)](http://www.veterinaryworld.org/Vol.13/May-2020/10.html)

[PDF \(http://www.veterinaryworld.org/Vol.13/May-2020/10.pdf\)](http://www.veterinaryworld.org/Vol.13/May-2020/10.pdf)

Research (Published online: 15-05-2020)

11. Sensitivity of polymerase chain reaction in the detection of rat meat adulteration of beef meatballs in Indonesia

G. Y. Suryawan, I. W. Suardana and I. N. Wandia
Veterinary World, 13(5): 905-908

[Abstract \(http://www.veterinaryworld.org/Vol.13/May-2020/11.html\)](http://www.veterinaryworld.org/Vol.13/May-2020/11.html)

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Research (Published online: 16-05-2020)

12. Semi-domesticated dogs as a potential reservoir for zoonotic hookworms in Bangkok, Thailand

Jutamas Wongwigkan and Tawin Inpankaew
Veterinary World, 13(5): 909-915

[Abstract \(http://www.veterinaryworld.org/Vol.13/May-2020/12.html\)](http://www.veterinaryworld.org/Vol.13/May-2020/12.html)

[PDF \(http://www.veterinaryworld.org/Vol.13/May-2020/12.pdf\)](http://www.veterinaryworld.org/Vol.13/May-2020/12.pdf)

Research (Published online: 18-05-2020)

13. Molecular detection and genetic variability of *Ehrlichia canis* in pet dogs in Xinjiang, China

Qiao Mengfan, Wang Lixia, Lei Ying, Ren Yan, Cai Kuojun, Zhang Jinsheng, Zhang Zaichao, Yu Weiwei, Peng Yelong, Cai Xuepeng, Qiao Jun and Meng Qingling
Veterinary World, 13(5): 916-922

[Abstract \(http://www.veterinaryworld.org/Vol.13/May-2020/13.html\)](http://www.veterinaryworld.org/Vol.13/May-2020/13.html)

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Research (Published online: 18-05-2020)

14. Biochemical and immunological investigation of fascioliasis in cattle in Egypt

Nani Nasreldin and Rania Samir Zaki
Veterinary World, 13(5): 923-930

[Abstract \(http://www.veterinaryworld.org/Vol.13/May-2020/14.html\)](http://www.veterinaryworld.org/Vol.13/May-2020/14.html)

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Alpha-lipoic acid ameliorates sodium valproate-induced liver injury in mice

Chrimawan Ardianto¹, Hijrawati Ayu Wardani, Nurrahmi Nurrahmi, Mahardian Rahmadi and Junaidi Khotib²

Department of Clinical Pharmacy, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60115, Indonesia.

Corresponding author: Chrimawan Ardianto, e-mail: chrimawan-a@ff.unair.ac.id

Co-authors: HAW: hijrawatiayuardani@gmail.com, NN: nurrahmi.siswanto20@gmail.com,

MR: mahardianr@ff.unair.ac.id, JK: junaidi-k@ff.unair.ac.id

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Abstract

Aim: This study examines the effect of alpha-lipoic acid (ALA) on sodium valproate-induced liver injury through histological features of mice liver tissue.

Materials and Methods: Mice were divided into three groups; (1) vehicle group, (2) sodium valproate group, and (3) sodium valproate-ALA group. The vehicle group was injected with saline intraperitoneal (i.p.) for 28 days. The sodium valproate group was injected with sodium valproate 300 mg/kg, i.p. daily for 2 weeks, after which the vehicle was administered daily until day 28. The sodium valproate-ALA group was injected with sodium valproate 300 mg/kg daily for 2 weeks before the administration of ALA 100 mg/kg i.p. until day 28. The mice were euthanized, and the liver was extracted for histopathological examination.

Results: Histopathological examination of the liver section of the vehicle group showed a normal structure of the liver. Two weeks after the administration of sodium valproate, histopathological examination showed an abnormal structure of the liver, with necrotic appearance and inflammatory cells. Moreover, treatment with ALA after the administration of sodium valproate notably ameliorated hepatic histopathological lesions and the liver structure corresponded to a normal liver structure.

Conclusion: ALA ameliorates sodium valproate-induced liver injury in mice.

Keywords: alpha-lipoic acid, drug-induced liver injury, histopathological, liver injury, sodium valproate.

Introduction

Epilepsy is one of the most common chronic neurologic disorders. Approximately 70 million people have epilepsy worldwide and approximately 90% of them are from developing regions [1]. Sodium valproate is a commonly prescribed antiepileptic drug used to treat various seizure disorders. Furthermore, severe side effects such as hepatotoxicity, pancreatitis, thrombocytopenia, and platelet aggregation are associated with valproate treatment [2]. The liver is the primary organ for the metabolism of many antiepileptic drugs and is subjected to drug-induced injury. The mechanism of hepatotoxicity remains unclear, and overproduction of reactive oxygen species (ROS) and compromised antioxidant capacity as a result of oxidative stress has been hypothesized to play a role in the etiology of toxicity. In addition, several studies have reported that valproate treatment is associated with oxidative stress [3].

However, efforts are being made to overcome drug-induced liver injury (DILI). To overcome the potential harmful effects of free radicals and to reduce the damage by oxidants, many antioxidants have been examined in trials as scavengers to stop the injury. Alpha-lipoic acid (ALA), one of the most effective antioxidants, is known to be involved in the cellular antioxidant system. ALA, known as thioctic acid or 1, 2-dithiolane-3-pentanoic acid ($C_8H_{14}O_2S_2$), is an essential cofactor in mitochondrial dehydrogenase reactions, soluble in water and lipid, and widely distributed in the cellular membrane, cytosol, and extracellular space [4]. Several investigations have reported the hepatoprotective effects of ALA [5-7]; however, there is a lack of information regarding the role of ALA in sodium valproate-induced liver injury.

This study examines the effect of ALA on sodium valproate-induced liver injury in mice observed through the histological features of the liver.

Materials and Methods

Ethical approval

All experiments were conducted at the Animal Research Laboratory of the Faculty of Pharmacy Universitas Airlangga, Surabaya, Indonesia, in accordance with the Guidelines for the Care and Use of Laboratory Animals issued by the National Institutes

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of Health revised in 1985. The Ethics Committee of Faculty of Veterinary Medicine Universitas Airlangga, Surabaya, Indonesia, approved the study protocol.

Materials

Sodium valproate powder was obtained from the Kalbe Farma pharmaceutical industry, Indonesia. ALA was obtained from Simex Pharmaceutical Indonesia in the form of a powder. Sodium valproate 300 mg/kg was diluted in a saline solution, and ALA 100 mg/kg was diluted in 50% propylene glycol solution.

Animals, experimental design, and treatments

Male ICR mice weighing between 25 and 30 g were used. The animals were housed in chip-bedded plastic cages at room temperature ($25^{\circ}\text{C}\pm 2^{\circ}\text{C}$) in a 12-h light/dark cycle at the Animal Research Laboratory of the Faculty of Pharmacy Universitas Airlangga. Free access to drinking water and standard chow food was provided to the mice until the end of the study. The mice were divided into three groups; (1) vehicle group, (2) sodium valproate group, and (3) sodium valproate-ALA group. The vehicle group was injected with saline intraperitoneal (i.p.) for 28 days. The sodium valproate group was injected with sodium valproate 300 mg/kg i.p. daily for 2 weeks, after which the vehicle was administered daily until day 28. The sodium valproate-ALA group was injected with sodium valproate 300 mg/kg daily for 2 weeks, before the administration of ALA 100 mg/kg i.p. until day 28.

Histopathological examination

At the end of the study, the mice were euthanized and the liver was extracted. Liver fragments were fixed in formalin at 10% and processed and embedded in paraffin. Further, 3 μm sections were made and subjected to hematoxylin and eosin staining. Under the optic microscope, the slides were examined and digital images were captured. The portal area, which is the most sensitive area in liver damage, was examined. The pattern of hepatocytes, infiltration of the inflammatory cells, and cell necrosis were observed.

Results

The histopathological examination of the liver section of the vehicle group showed a normal structure of the liver observed at 100 \times and 400 \times (Figures-1 and 2). Two weeks after the administration of sodium valproate, histopathological assessment exhibited an abnormal liver structure at 100 \times (Figure-3) and 400 \times (Figure-4). In the liver architecture, there were partial distortions, accompanied by focal vacuolar degenerative changes in hepatocytes. The focal areas of necrosis with the inflammatory cells were detected. In addition, the scattered focal aggregates of the inflammatory cells were observed in the portal areas and the area between hepatocytes. Moreover, the result showed mild degeneration of hepatocytes followed by widening of sinusoids and increase in Kupffer cells. Besides, the majority of the hepatocytes showed vacuolation, accompanied

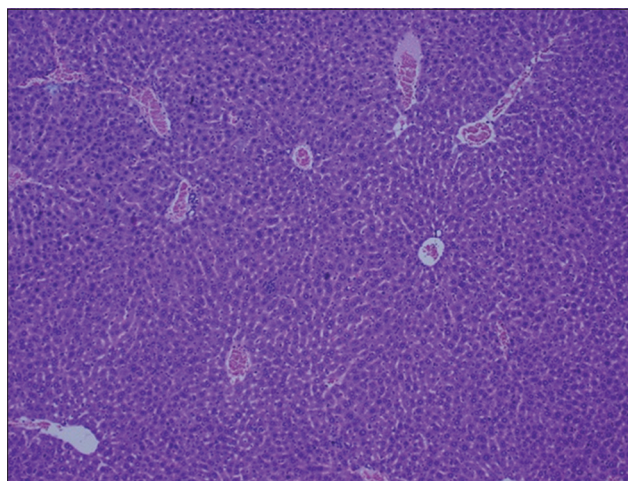


Figure-1: The representative figure of liver section showed normal structure in the vehicle group (100 \times).

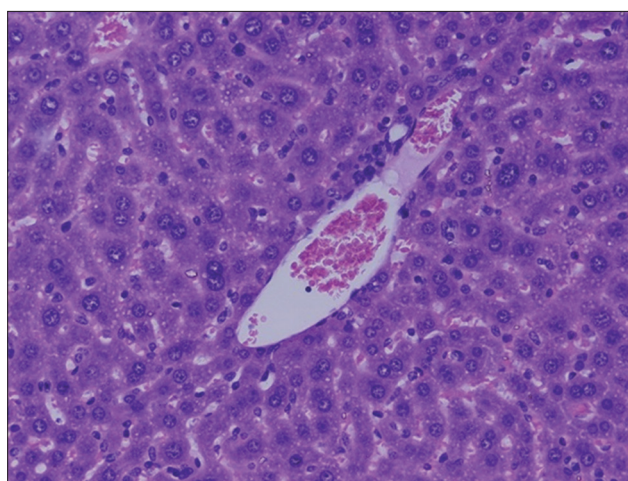


Figure-2: The representative figure of liver section showed normal structure in the vehicle group (400 \times).

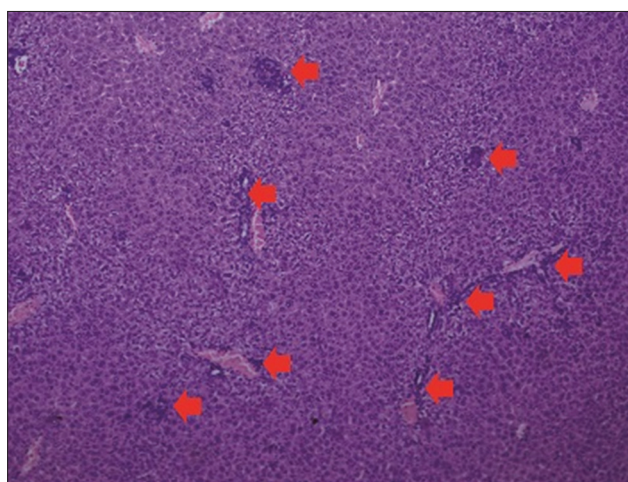


Figure-3: The representative figure of liver section showed abnormal structure in the sodium valproate group (100 \times). Necrosis and inflammatory cells infiltration were observed.

by variation in the size and shape of the nucleus. Furthermore, hypertrophied nuclei were observed. Treatment with ALA 100 mg/kg considerably ameliorated the hepatic histopathological lesions observed

at 100× (Figure-5). Moreover, our findings showed that the liver structure corresponded to a normal liver structure observed at 400× (Figure-6).

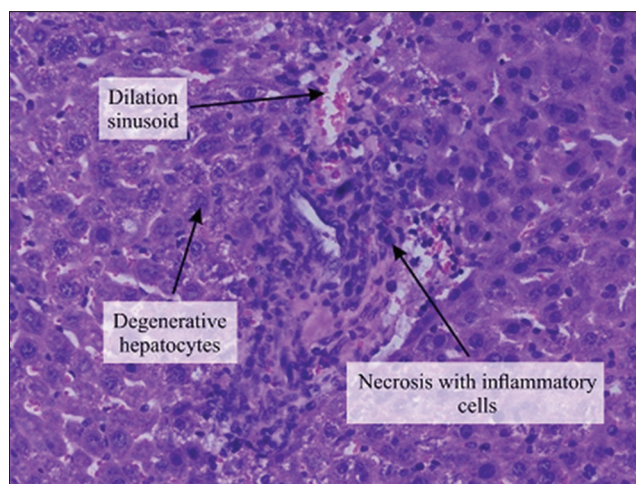


Figure-4: The representative figure of liver section showed abnormal structure in the sodium valproate group (400×). Necrosis with inflammatory cells infiltration, dilation of sinusoid, and degenerative hepatocytes were observed.

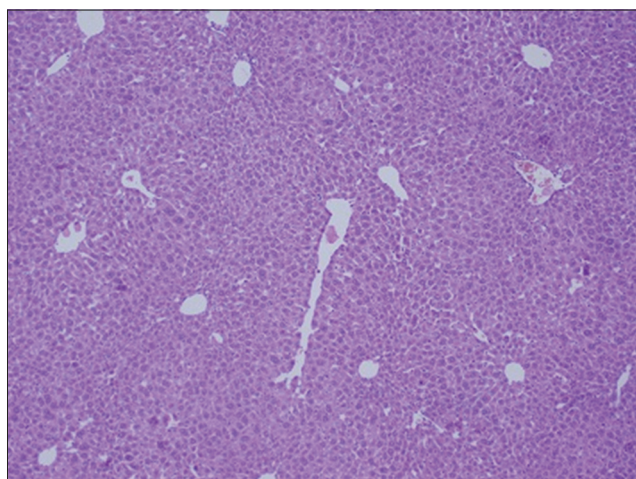


Figure-5: The representative figure of liver section showed normal structure in the sodium valproate-alpha-lipoic acid group (100×).

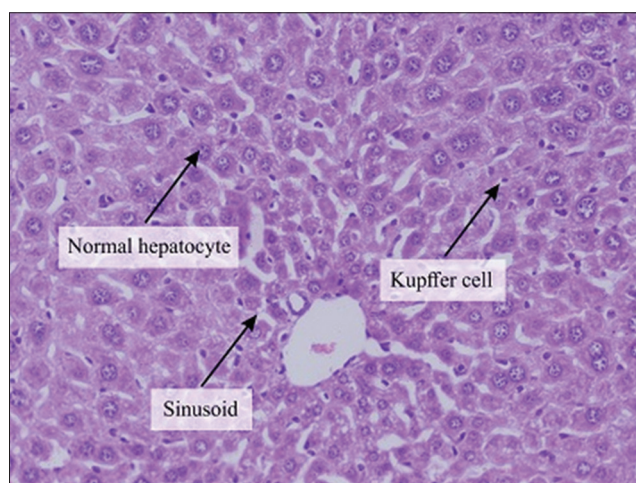


Figure-6: The representative figure of liver section showed normal structure in the sodium valproate-alpha-lipoic acid group (400×).

Discussion

Sodium valproate is known to cause DILI. The clinical feature of DILI has been reported to exhibit a specific pattern in clinical data and liver histology. Hepatocyte necrosis followed by marked inflammatory activity is the most common pattern observed in DILI and has become the gold standard in evaluating the condition [4]. The histopathological examination of the liver sections in the present study showed that the vehicle group showed a normal structure of the liver. However, the sodium valproate group exhibits impairment in several areas of the liver. Histopathological features after daily administration of sodium valproate 300 mg/kg i.p. for 2 weeks showed an abnormal structure of the liver. The sodium valproate group exhibited necrosis followed by the aggregation of the inflammatory cells, mild degeneration of hepatocytes with widening of sinusoids, and increase in Kupffer cells. The increase in Kupffer cells reflected the increase in the inflammatory process; however, the pathogenesis of such hepatotoxicity remains unclear. Several mechanisms have been proposed for valproate-induced hepatotoxicity, including reactive metabolites of valproate [8,9]. In addition, the involvement of carnitine deficiency [10,11], hyperammonemia [5], and oxidative stress or enhanced production of ROS has been reported [3,12]. This is supported by our findings showing that there was a considerable increase in serum ALT levels in the sodium valproate group compared with the vehicle group but not in serum AST levels (data not shown). ALT has been considered a reliable and sensitive marker of liver disease, and elevated serum ALT levels can effectively identify an ongoing liver disease [13].

The present investigation showed that treatment with ALA 100 mg/kg after sodium valproate-induced liver injury for 2 weeks notably decreases the hepatic lesions. The histological features showed that ALA preserved the normal structure of the liver under valproate administration. The previous investigations reported a hepatoprotective effect of ALA [5-7]. A study reported that ALA protects hepatocytes by suppressing hepatic oxidative stress as well as down-regulating the expression of hepatic pro-inflammatory cytokines, iNOS, and NF- κ B [14]. However, one study reported that in *in vivo*, lipoic acid was most likely associated with the inhibition of β -oxidation or glucuronidation, the two dominant metabolic processes of valproate [15]. Further studies are needed to clarify this issue.

Conclusion

The present results indicated that ALA ameliorated sodium valproate-induced liver injury in mice. For the 1st time, the present study provided direct evidence of the *in vivo* efficacy of ALA in the treatment of valproate-induced liver injury. Further research is needed to clarify the protective mechanism of ALA

in valproate-induced liver injury and the prospects of ALA in clinical use for such indication.

Authors' Contributions

CA, HAW, and JK designed the plan of work. CA, HAW, MR, and NN performed laboratory investigation. CA, HAW, MR, and JK participated in draft and revision of the manuscript. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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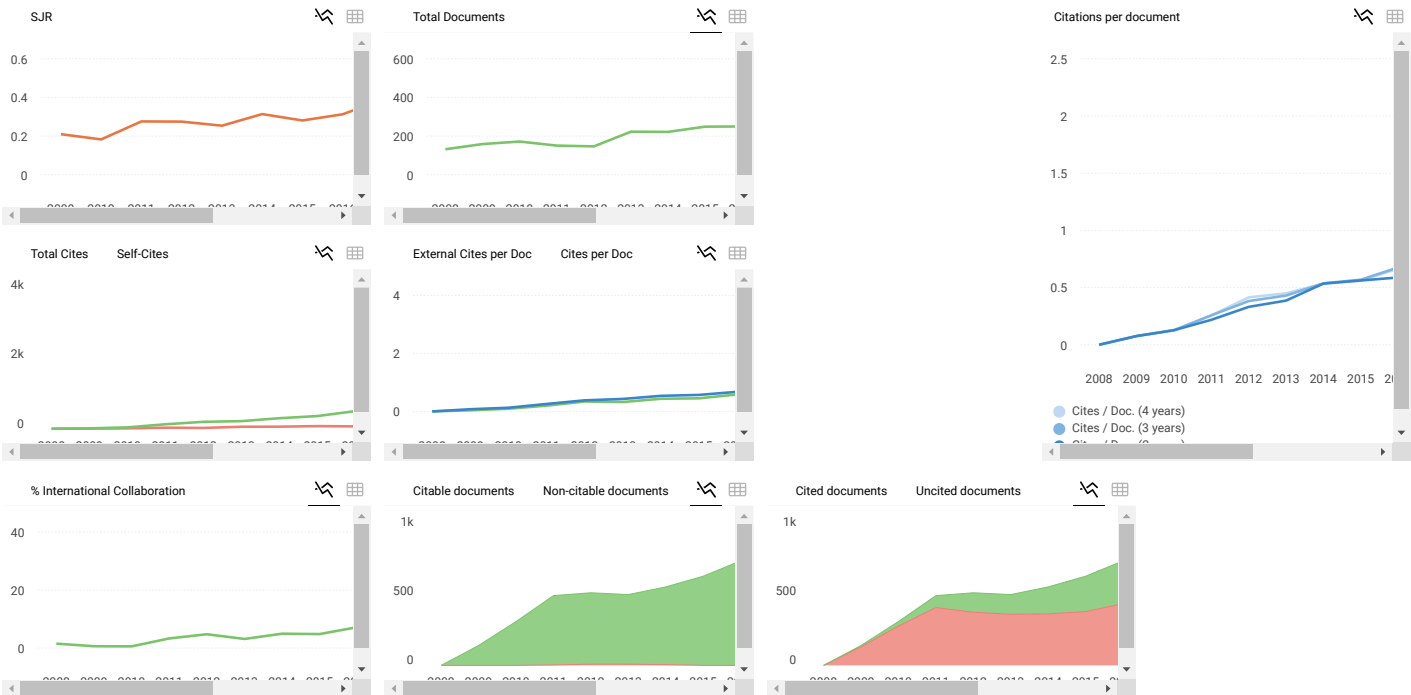
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