



PRELIMINARY SURVEY VISIT

AREA V RESEARCH

D. PUBLICATION AND DISSEMINATION

D.1. Evidence of Publication and Dissemination of Research Results



Research and Development Office



As the University **Research and Development Office (RDO)** upholds the status of excellence in research and development, it continues to be the catalyst of technology development and information. The office research track has been focusing into different themes such as access to education and literacy; environmental protection &

conservation; food security and poverty alleviation; health and proper nutrition; engineering and technology; preservation of arts, culture, language and literature; promotion of peace, gender equality and development; and halal.

R DO is primarily under the umbrella of the **Vice-President for Research, Development and Extension**, whose prime function is to monitor and supervise students' researches, at the same time evaluate and monitor research projects conducted by full-time and faculty researchers. In addition, the office is mandated to:

- Improve knowledge products through collaborative and interdisciplinary R & D activities that are anchored on the University's vision/mission;
- Do research, generate/develop new technologies and information as solutions to improve and address local, regional and national concerns and related issues;
- Develop mechanisms to ensure that the research results are effectively and efficiently delivered and disseminated to the target clientele for appropriate utilization and commercialization; and
- Support resource generation efforts for continuous and sustainable R & D programs that are geared toward ensuring money availability and food security, global competitiveness and socio-cultural and socio-economic responsiveness that eventually improve the quality of life of the clienteles.

 STAFF



Lydia C. Pascual
Director



Jayson S. Baltazar
Education Program Specialist I



Leizl Gray T. Oria
Educational Program Specialist I



Organization

Locally-Funded Research

Locally-Funded Research

2025 2024 2023 2022 2021 2020 2019 2018 2017 2016 2015

2025

Mobile App and Sensor Integration for NPK Levels Testing in Real-Time (MoNITR)

DHEALYN DECEE V. SABIT, REZIN G. CABANTUG, CARMEE LYN B. PAYLANGCO

USM FUND 101 – Php. 100,000.00

Micro Matters: Assessing the Potential of Developing Microcredential Programs in the University of Southern Mindanao

ROMIEL JOHN BASAN

USM FUND 101 – Php. 165,000.00

Intelligent Feedback Management System for University of Southern Mindanao Service Personnel Using Advanced Software Development Framework and Artificial Intelligence Technologies

JOSEPH LORILLA

USM FUND 101 – Php. 200,000.00

Modeling Afforestation Sites: A Decision Support Tool for Sustainable Land Management

JOHN ARIES TABORA

USM FUND 101 – Php. 250,000.00

Generalized E-torsion Graph; Forcing Subsets for perfect Roman dominating sets in graphs; Convex Graph induced by a Function and a Finite Set

LEONARD M. PALETA, JUPITER G. PILONGO, PHILIP LESTER P. BENJAMIN

USM FUND 101 – Php. 107,000.00

Seeds of Innovation: USM's Pursuit of Breakthroughs in Cacao, Rubber, Corn, and Coffee Research

JAYSON BALTAZAR, LEIZL GRAY ORIA, MARRY GRACE BALBUENA, JOHN ALDRIN SANAMA

USM FUND 101 – Php. 104,400.00

Digital Streamlining of USM RDE Initiatives**LEIZL GRAY ORIA, JAYSON BALTAZAR**

USM FUND 101 – Php. 72,000.00

Metrics Of Success Of R&d Projects In Usm; Input For Data-driven Performance Review**KHARLO SUBRIO, RAHIMA CABUNTO**

USM FUND 101 – Php. 18,000.00

Tracer Study For 2020-2023 Graduates Of USM**KHARLO SUBRIO**

USM FUND 101 – Php. 46,127.00

AIIDE INITIATIVE - AI Instructional Delivery , and Ethics: Harnessing the Power of AI for Educational Advancement**JOHN ALDRIN SANAMA**

USM FUND 101 – Php. 65,250.00

Syllabus OBEdition: Analyzing General Education and Selected Major Course Syllabi with Insights from Established Frameworks to Refine Outcome-Based Syllabus at USM**RAHIMA CABUNTO**

USM FUND 101 – Php. 45,000.00

Syllabus OBEdition: Analyzing General Education and Selected Major Course Syllabi with Insights from Established Frameworks to Refine Outcome-Based Syllabus at USM**LEIZL GRAY ORIA, LYDIA C. PASCUAL, JASMIN A. PECHO, MA. TEODORA N. CABASAN**

USM FUND 101 – Php. 150,000.00

UNIVERSITY OF SOUTHERN MINDANAO AGRICULTURAL RESEARCH AND DEVELOPMENT CENTER (USMARDC)**A Multifaceted Approach on Diseases Associated with Dragon Fruit and its Control Measures under Kabacan Condition****JASMIN A. PECHO, MARRY GRACE S. BALBUENA**

USM FUND 101 – Php. 150,000.00

Breeding and Yield Trial of Herbicide Tolerant Multi-Cross Hybrids**JESSIE G. ELARDE, NENITA E. OLERO, EFREN E. MAGULAMA**

USM FUND 101 – Php. 253,809.00

Comprehensive Evaluation of Durian Clone Yields and Pest Dynamics in the USM Clonal Garden**JANICE M BANGOY, JASMIN PECHO**

USM FUND 101 – Php. 200,000.00

Enhancing Hybridization and Evaluation of Popular Cultivar of Musang King and Black Thorn of Durian

SHEENA B. LUCENA, JURHAMID C. IMLAN, MARRY GRACE BALBUENA, JASMIN A. PECHO

USM FUND 101 – Php. 300,000.00

Project: Impact of USMs Pre-Storage Chemical Treatment on Corn Seed Storability and Germination

JANICE M BANGOY, EFREN MAGULAMA, NENITA OLERO

USM FUND 101 – Php. 200,000.00

BREADFRUIT MICROPROPOGATION: A Step Towards a More Sustainable and Resilient Food Security

MARRY GRACE S. BALBUENA, JURHAMID IMLAN

USM FUND 101 – Php. 200,000.00

Validation of Herbicide Resistant Corn Lines Through Molecular and Morphological Evaluation

MARRY GRACE S. BALBUENA, JESSIE ELARDE, JOHN ALDRIN SANAMA

USM FUND 101 – Php. 200,000.00

PHILIPPINES INDUSTRIAL CROPS RESEARCH INSTITUTE

Program: Hybridization of Rubber for the Development of High- Yielding and Disease-Resistant Clones (YEAR 2)

JANICE M BANGOY, JASMIN A PECHO, SHEENA B LUCENA, MARRY GRACE BALBUENA

USM FUND 101 – Php. 300,000.00

Plugging into Efficiency: Innovations in Soil Electrical Conductivity for Enriched Nutrient Availability and Crop Productivity

REZIN CABANTUG

USM FUND 101 – Php. 150,000.00

GIS-Aided Assessment of the Pest, Disease, and Biochemical Status of the USM Agricultural Research and Development Center (USMARDC) of the existing Land Use Utilization and Future Management

REZIN G. CABANTUG, JURHAMID C. IMLAN, JANICE M. BANGOY, JASMIN A. PECHO, MARRY GRACE S. BALBUENA

USM FUND 101 – Php. 150,000.00

Commercialization and Opportunities for Market Performance and Enhanced Technologies in High-Value Crops at the University of Southern Mindanao

JALALODEN B. MAROHOM, REZIN G. CABANTUG, HAREM R. ROCA

+ USM Fund 101



Organization

Locally-Funded Research

Locally-Funded Research

2025 2024 2023 2022 2021 2020 2019 2018 2017 2016 2015

2024

Characterization of Palm Oil Products Using GC-MS And FTIR and its Potential Application for Bio-Plasticizers

MARIVIC D. CANDARI, QUEENNIE L. RUFINO, NIKKI JANE S. BENITO

USM FUND 101 – Php. 300,000.00

USMGEO: University Spatial Mapping and Geographic Information System Portal

KATHLEEN MAE B. ALUCILJA, EUGENE RANJO, RENEL M. ALUCILJA

USM FUND 101 – Php. 306,000.00

Mapping the Future Plate: Evaluating the Spatiotemporal Dynamics of Meso-Level Food Security (MFS) of Cotabato Province in Changing Climate

ROMIEL JOHN BASAN

USM FUND 101 – Php. 153,000.00

Molecular Identification and Control of Brown Leaf Malady of Selected Fruit Crops

JASMIN A. PECHO, MARRY GRACE S. BALBUENA, CLARK I MAARAT, TAMIE C. SOLPOT

USM FUND 101 – Php. 250,000.00

Phenotypic Evaluation of Multi-Cross Hybrids and Synthetic with Tolerance to Herbicide

JURHAMID IMLAN, SHEENA B. LUCENA, EFREN E. MAGULAMA

USM FUND 101 – Php. 250,000.00

UNIVERSITY OF SOUTHERN MINDANAO AGRICULTURAL RESEARCH AND DEVELOPMENT CENTER (USMARDC)

Phenotypic Evaluation of Multi-Cross Hybrids and Synthetic with Tolerance to Herbicide

JESSIE G. ELARDE, NENITA E. OLERO, EFREN E. MAGULAMA, EDWARD A. BARLAAN

USM FUND 101 – Php. 163,404.00

Breeding of Corn Varieties with Climate Resilience: Drought Tolerance

NENITA E. OLERO

USM FUND 101 – Php. 190,456.00

Breeding Of White OPVS, Glutinous and Sweet Corn for Herbicide Tolerance and Corn Borer Resistance

NENITA E. OLERO, JESSIE G. ELARDE, EFREN E. MAGULAMA, MONAIRA SUMAIL

USM FUND 101 – Php. 223,126.00

Optimization and Validation of USM Developed Bio-stimulant for Dragon Fruit Production

JASMIN A. PECHO

USM FUND 101 – Php. 120,000.00

PHILIPPINES INDUSTRIAL CROPS RESEARCH INSTITUTE

Anti-Inflammatory and Therapeutic Benefits of Liniment Formulations Extracted from Herbs and Spices

REZIN G. CABANTUG, ABUBAKAR A. MURRAY

USM FUND 101 – Php. 130,280.73

Screening for Resistance and Fungicide for Pestalotiopsis Leaf Spot disease of Coffee

BERNADITH BORJA, TAMIE SOLPOT

USM FUND 101 – Php. 100,000.00

Hybridization of Rubber (Hevea brasiliensis) Towards Development of High-Yielding and Disease Resistant Clones

JANICE BANGOY, JASMIN PECHO, SHEENA LUCENA, NOE MAMON, MARRY GRACE BALBUENA

USM FUND 101 – Php. 100,000.00

Development of Potential coffee and cacao Agri-Product Based beverages

LORELYN JOY N. TURNOS-MILAGROSA, ABUBAKAR A. MURRAY, SEDRA MURRAY, MARK AL-JAMIE J. MUTTULANI

USM FUND 101 – Php. 100,000.00

Stabdardization of Organic Herbicide Formulations Towards Enhanced Weed Mangement for Sustainable Farming

REZIN G. CABANTUG, JOSEPH CASTILLO

USM FUND 101 – Php. 100,000.00

FUTURES' THINKING

University of Southern Mindanao Futures Thinking for Food Security and Health, Systems, Innovations and Sustainability

FRANCISCO GIL N. GARCIA, MA. TEODORA N. CABASAN, EDWARD A. BARLAAN

USM FUND 101 – Php. 2,000,000.00

MINSUPALA

Water Quality Assessment and Characterization of Liguasan Marsh in Cotabato Province: A Comprehensive Analysis of Physico-Chemical Parameters, Heavy Metal Contamination, and Biological Assessment

LYDIA C. PASCUAL, DEBBIE MARIE B. VERZOSA, HANNA LOU B. DIEZ, LEMUEL JON P. GABUAY

USM FUND 101 – Php. 1,594,296.00

Characterization of Inland Capture Fishery and Rapid Assessment of Fish Abundance and Diversity of Liguasan Marsh in Cotabato Province

LEONILA V. PAPALID, CYREX LOUIE JAY E. GAURAN, REGINE N. LENIS

USM FUND 101 – Php. 1,201,777.75

Assessment and Modelling of Land Use, Land Cover Change and its Impact on Soil Quality, Hydrologic Patterns, and Sedimentation Rates in the Libungan-Alamada Watershe

JENNET R. MAG-ASO, REZIN G. CABANTUG, ANDREA KATE C. CASTAÑEDA

USM FUND 101 – Php. 1,135,740.75

Assessment of Socioeconomic Status of Communities in the Liguasan Marsh in Cotabato Province

FRANCISCO GIL N. GARCIA, ROMIEL JOHN P. BASAN, LADY ANN R. TURIAGA

USM FUND 101 – Php. 1,068,184.50

 Externally-Funded Research

 College Research Coordinators

Vision

Quality and relevant education for its clientele to be globally competitive, culture-sensitive and morally responsive human resources for sustainable development.

Mission

Help accelerate socio-economic development, promote harmony among diverse communities and improve quality of life through instruction, research, extension and resource generation in Southern Philippines.

Our Address

Bai Matabay Plang Avenue
Poblacion, Kabacan
Cotabato 9407
Philippines

Visitor Counter

Today's Visitors:
3
Last 7 Days Views:
123
Total Visitors:
2,809,327

Data Protection

About
Security Breach Incident
Students, Alumni & Prospective
Students
Employees
Visitors
Website Users



Organization

Locally-Funded Research

Externally-Funded Research

Externally-Funded Research

2025 2024 2023 2022 2021 2020 2019 2018

2025

MAINSTREAMING AANR TECHNOLOGIES AND INFORMATION IN MINDANAO THROUGH SOXARDEC'S PARTICIPATION IN RSTW AND OTHER LOCAL EXHIBITS

Gelyn V. Amilbahar

DOST-PCAARRD Budget: Php 344,250.00

IARC: IMPACT ASSESSMENT OF DOST-PCIEERD REGIONAL CONSORTIA IN THE PHILIPPINES

Romiel John P. Basan

DOST-PCIEERD Budget: Php 3,032,523.20

ISOLATION OF LACTIC ACID BACTERIA FROM JOB'S TEARS (COIX LACRYMA-JOBI) AND TWO FORAGES AND THEIR UTILIZATION AS INOCULANT IN SILAGE PRODUCTION FOR HALAL RUMINANT FEED

Josephine R. Migalbin

MOST – BARMM Budget: Php 1,050,038.00

PEST AND DISEASES ASSOCIATED WITH INFLORESCENCE AND IMMATURE NUTS AFFECTING HYBRID PRODUCTION

Tamie C. Solpot

DOST-PCAARRD Budget: Php 5,000,000.00

INFLUENCE OF PLANT GROWTH REGULATORS TO IMPROVE HYBRID NUT PRODUCTION IN DWARF COCONUTS

Mark Al-jamie Mutullani

DOST-PCAARRD Budget: Php 5,000,000.00

DEVELOPMENT OF APPROPRIATE TREATMENTS TO COCONUT INFLORESCENCE TO IMPROVE HYBRIDIZATION OPERATIONS

Lorelyn Joy N. Turnos – Milgarosa

DOST-PCAARRD Budget: Php 5,000,000.00

DEVELOPMENT OF SOIL AND WATER MANAGEMENT GUIDE TO IMPROVE COCONUT HYBRID NUT PRODUCTIVITY AND QUALITY

Mel Chrisel A. Sales

DOST-PCAARRD Budget

PROGRAM: DEVELOPING SOIL KNOWLEDGE, INFORMATION AND CAPACITY TO IMPROVE THE PRODUCTIVITY AND SUSTAINABILITY OF KEY CROPPING SYSTEMS IN THE PHILIPPINES

Ardniel A. Baladjay, Rezin G. Cabantug

DOST-PCAARRD Budget: Php. 36,808,050.00

PRESERVING MAGUINADANON TRADITIONAL MUSIC THROUGH LEARNING PACKAGES, DIGITAL APPS, AND IEC MATERIALS

Debbie Marie B. Verzosa, Saima M. Andil, Jikiri M. Entol

MOST – BARMM Budget: Php. 1,273,429.85

USM CENTER FOR FLOOD/ LANDSLIDE PREPAREDNESS AND MITIGATION

Christopher Benito

DOST-PCIEERD Budget: Php 4,992,784.00

HARNESSING RHIZOBACTERIA AND ACTINOMYCETE'S POTENTIAL AS BIOFERTILIZER AND BIOCONTROL INPUTS SUPPORTIVE OF STRENGTHENING THE ORGANIC AGRICULTURE INDUSTRY (HARBIS-ORGANIC)

Mel Chrisel A. Sales, Ma. Teodora N. Cabasan, and Neil Pep Dave N. Sumaya

DA-BAR Budget: Php 2,569,587.50

HEIRIT: ESTABLISHMENT OF USMART TECHNOLOGY BUSINESS INCUBATOR - A DOST FUNDED TBI

Jalaloden B. Marohom, Maricel G., Dayaday, Danilyn A. Flores, Nerissa G. Dela Viña, Romiel John P. Basan

DOST-PCIEERD Budget: Php 13,400,000.00

SATELLITE SERDAC: ESTABLISHMENT OF SATELLITE SOCIO-ECONOMIC AND DATA ANALYTICS CENTER (SERDAC) AT THE UNIVERSITY OF SOUTHERN MINDANAO TO STRENGTHEN CAPACITY IN SOCIO-ECONOMIC RESEARCH AND ANALYTICS IN THE REGION *(on-going)*

Romiel John P. Basan, Roy Gacus, Helen Macailling, Kathleen Ivy Bolotaolo, Metche Anne Logronio

DOST-PCAARRD Budget: Php 4,160,007.30

REGIONAL AGRI-AQUA INNOVATION SYSTEM ENHANCEMENT (RAISE) PROGRAM IN SOCCSKSARGEN *(on-going)*

Program Leader: **Josephine R. Migalbin**

+ DOST-PCAARRD Budget

DEVELOPING SUSTAINABLE AGRICULTURAL ENVIRONMENT THROUGH THE ORGANIZATION OF DURIAN PRODUCERS IN MINDANAO, PHILIPPINES *(on-going)*

Mel Chrisel Sales

CAMP ASIA, Inc/ KOICA

SMART CACAO BUDWOOD NURSERY AND GREENHOUSE FOR PRODUCTION OF HIGH-QUALITY PLANTING MATERIALS *(on-going)*

Edward A. Barlaan, Maricel Dayaday, and Renel M. Alucilja

DOST-PCAARRD: Php. 24,176,622.80

 College Research Coordinators

Vision

Quality and relevant education for its clientele to be globally competitive, culture-sensitive and morally responsive human resources for sustainable development.

Mission

Help accelerate socio-economic development, promote harmony among diverse communities and improve quality of life through instruction, research, extension and resource generation in Southern Philippines.

Our Address

Bai Matabay Plang Avenue
Poblacion, Kabacan
Cotabato 9407
Philippines

Visitor Counter

Today's Visitors:
3
Last 7 Days Views:
123
Total Visitors:
2,809,327

Data Protection

About
Security Breach Incident
Students, Alumni & Prospective
Students
Employees
Visitors
Website Users

"Your Partner in Academic Excellence and Leadership Development."

© 2026 University of Southern Mindanao. All Rights Reserved.



[Home](#)[About](#)[Administration](#)[Academics](#)[RD & E](#)[RGE5](#)[Student](#)[Alumni](#)[Media](#)

Research Publication Services Office

[Home](#) > [Research, Development & Extension](#) > [Office of the Vice-President Research, Development and Extension](#) > [Research Publication Services Office](#)



The RDE envisions that USM will manage and publish journals that are included in international indexes such as Web of Science and Scopus. The **Research Publication Services Office (RPSO)** handles peer-reviewed journals published by USM colleges or institutes. These include the **Journal of Agricultural Research, Development, Extension and Technology (JARDET)** and **Studies in Natural and Applied Sciences (SNAPS)**. All journals can be accessed through USM's **online journal website**. All USM peer-reviewed journals publish articles not only from USM but also from contributors elsewhere.

The RPSO also facilitates the implementation of Publication and Invention/Intellectual Property Awards with corresponding financial incentives. This is to encourage faculty and fulltime researchers to publish research in peer-reviewed or ISI/SCOPUS indexed journals, file patents for inventions or file for copyrights.

Publication Incentives

Prior to submission, authors are strongly recommended to inform the URDEC through the RPSO of their intent to publish through the **Application Form for Intent to Publish** (URDEC Resolution No. 08 s2020, Policy Guidelines in the Paper Publication and Provision of Monetary Incentive/Awards). Upon approval of the intent to publish, the potential authors will receive a notice to proceed, and submit their article to their indicated journal. Potential authors with disapproved applications will also be informed accordingly.

After publication, authors shall inform the URDEC through the RPSO of their publication and email their full paper to rpso@usm.edu.ph for evaluation for publication incentive.

The application for intent to publish was put in place to provide guidance to potential authors, and to assist the RPSO set targets for the number of publications and monitor submissions to high quality journals.

 STAFF

Florence Roy P. Salvaña


RPSO Director



Hannah Queen C. Pasco

Editorial Staff

**Journal of Agricultural Research, Development, Extension and
Technology (USM Journals Online)** 




UNIVERSITY OF SOUTHERN MINDANAO

JOURNALSONLINE
Home | Call For Papers | News

Search
Advanced Search

JARDET

Journal of Agricultural Research,
Development,
Extension, and Technology



SNAPS
Studies in Natural and Applied Sciences

Research and Development Office | University of Southern Mindanao
<https://journals.usm.edu.ph> | rpo@usm.edu.ph

Recipients of Publication Incentives

 **PUBLICATIONS**

All Publications
Biology
Environmental Science
Medicine
Liberal Arts and Social Sciences
Agriculture
Veterinary Medicine
Engineering
Mathematics
Chemistry
Business and Economics

Risks, hazards, harm and risk analysis: a brief introductory overview for veterinarians

Jan Clyden B. Tenorio

Emerging zoonotic infectious diseases: a folly of human development

Jan Clyden B. Tenorio

Vanguards of food safety and security: Acknowledging the humble veterinarian and one health

Jan Clyden B. Tenorio

Monsters in our food: Foodborne Trematodiasis in the Philippines and beyond

Jan Clyden B. Tenorio and Elizabeth C. Molina

A survey of forage-related toxicities in goats in Region XII, Philippines

Florence Roy P. Salvaña, Elma G. Sepelagio, Carlito B. Sanchez, LB Cardenas

Anticoccidial Activity of Coleus aromaticus Leaves Extract in Naturally Infected Goats

Vyrnjelle J A. Gonzaga , Josephine R. Flores, Elsa A. Gonzaga, Loveille Jun A. Gonzaga

Performance of Broiler Chickens Fed Diets with Different Levels of Mangosteen (Garcinia mangostana Linn.) Rind Powder

Julius Jerome G. Ele, Josephine R. Migalbin, Elma G. Sepelagio and Peter Grearne F. Lacia

Effects of Slaughter Positions on Catecholamine, Blood Biochemical and Electroencephalogram Changes in Cattle Restrained Using a Modified Mark IV Box

Jurhamid Columbres Imlan, Ubedullah Kaka, Yong-Meng Goh, Zulkifli Idrus, Elmutaz Atta Awad, Ahmed Abubakar Abubakar, Tanbir Ahmad, Hassan N. Quaza Nizamuddin, and Awis Qurni Sazili

Physiological and Electroencephalogram Responses in Goats Subjected to pre- and during Slaughter Stress

Razlina Raghazli, Azalea-Hani Othman, Ubedullah Kaka, Ahmed A. Abubakare, Jurhamid C. Imlan, Hazilawati Hamzah, Awis Q.Sazili, Yong-MengGo

Physiological and Electroencephalographic Changes in Goats Subjected to Transportation, Lairage, and Slaughter

Azalea-Hani Othman, Yong Meng Goh, Noordin Mohamed Mustapha, Razlina Raghazli, Ubedullah Kaka, Jurhamid Columbres Imlan, Ahmed Abubakar Abubakar, Rasedee Abdullah

Effects of Stocking and Transport Conditions on Physicochemical Properties of Meat and Acute-Phase Proteins in Cattle

Ahmed A. Abubakar, Idrus Zulkifli, Yong M. Goh, Ubedullah Kaka, Azad B. Sabow, Jurhamid C. Imlan, Elmutaz A. Awad, Azalea H. Othman, Razlina Raghazli, Helen Mitin, and Awis Q. Sazili

The Effects of Stocking Density and Distances on Electroencephalographic Changes and Cortisol as Welfare Indicators in Brahman Crossbred Cattle

Ahmed A Abubakar, Idrus Zulkifli, Yong M Goh, Ubedullah Kaka, Azad B Sabow, Elmutaz A Awad, Jurhamid C Imlan, Azalea H Othman, Razlina Raghazli, Helen Mitin, Awis Q Sazili

Effects of Slaughter Knife Sharpness on Blood Biochemical and Electroencephalogram changes in Cattle

Jurhamid C. Imlan, Ubedullah Kaka, Yong-Meng Goh, Zulkifli Idrus, Elmutaz Atta Awad, Ahmed Abubakar Abubakar, Tanbir Ahmad, Hassan N. Quaza Nizamuddin, Awis Qurni Sazili

Campylobacter jejuni from farm to fork: Campylobacteriosis and chicken meat

Jan Clyden B. Tenorio, Vrenelie II D. Flores

Anthelmintic resistance of gastro-intestinal nematodes to albendazole, levamisole and ivermectin in Murrah Buffaloes

Khan L. Junatas, Elizabeth C. Molina

Schistosoma japonicum in the Philippines: Its epidemiology, diagnostics, control, and elimination

Jan Clyden B. Tenorio, Elizabeth C. Molina

Challenges in Goat Production Faced by Halal Goat Raisers in Region XII (SOCCSKSARGEN), Philippines

Carlito B. Sanchez, Florence Roy P. Salvaña, Elma G. Sepelagio, L.B. Cardenas

A new record of ethno-veterinary practice using Tinospora rumphii Boerl. among goat raisers in Southern Mindanao, Philippines

Florence Roy P. Salvaña, Elma G. Sepelagio and Carlito B. Sanchez

Evaluation of Selected Slaughterhouses and Parasites of Slaughtered Livestock in Cotabato Province, Mindanao, Philippines

Cyrelle M. Besana and V.G.V. Paller

Vision

Quality and relevant education for its clientele to be globally competitive, culture-sensitive and morally responsive human resources for sustainable development.

Mission

Help accelerate socio-economic development, promote harmony among diverse communities and improve quality of life through instruction, research, extension and resource generation in Southern Philippines.

Our Address

Bai Matabay Plang Avenue
Poblacion, Kabacan
Cotabato 9407
Philippines

Visitor Counter

Today's Visitors:
3
Last 7 Days Views:
123
Total Visitors:
2,809,327

Data Protection

About
Security Breach Incident
Students, Alumni &
Prospective Students
Employees
Visitors
Website Users

"Your Partner in Academic Excellence and Leadership Development"
© 2026 University of Southern Mindanao. All Rights Reserved.



List of Published Research of the College of Veterinary Medicine

Title	Author/s	Year of Publication	Journal	Indexing
1. Effectiveness of Drug-Based Interventions and Integrated Community Strategies for Soil-Transmitted Helminth Control in Southeast Asia: A Systematic Review	Jan Clyden B. Tenorio, Alok Kafle, Muhammad Fikri Heikal, Prasert Saichua, Sutas Suttiaprapa	2026	Infectious Microbes & Diseases	Scopus
2. Unraveling the Mechanisms of Benzimidazole Resistance in Hookworms: A Molecular Docking and Dynamics Study	Jan Clyden B Tenorio, Muhammad Fikri Heikal, Alok Kafle, Mark Andrian B Macalalad, Fredmoore L Orosco, Prasert Saichua, Sutas Suttiaprapa	2025	Journal of Genetic Engineering and Biotechnology	Scopus
3. Drug resistance in parasites: A review of mechanisms, drivers, and mitigation strategies	Jan Clyden Tenorio	2025	Microbes and Infectious Diseases	Scopus
4. Molecular docking and dynamics as a tool to study benzimidazole resistance in helminths: A scoping review	Jan Clyden Tenorio	2025	Journal of Livestock Science	Web of Science
5. Addressing schistosomiasis in the Philippines: Need for a coordinated intersectoral effort	Jan Clyden B Tenorio	2025	Asian Pacific Journal of Tropical Medicine	Scopus
6. Opisthorchis viverrini Helminth Defense Molecule: Structural features, molecular interactions, and dual immunomodulatory roles	Muhammad Fikri Heikal, Kamonrut Kongpha, Alok Kafle, Jan Clyden Tenorio, Sujittra Chaiyadet, Panupong Mahalapbutr, Kampeebhorn Boonloh, Krajang Talabnin, Thewarach Laha, Prasert Saichua, Sutas Suttiaprapa	2025	Acta Tropica	Scopus

7. A multifaceted approach to combatting the emergence of drug-resistant helminths	Jan Clyden B. Tenorio	2025	One Health Bulletin	CABI and EBSCO
8. Can resistant <i>Ancylostoma ceylanicum</i> arise and spread between humans and animals?	Jan Clyden B. Tenorio	2025	Microbes and Infectious Diseases	Scopus
9. Ultrastructural evidence of telocytes in the embryonic chick heart	Khan Lamanero Junatas, Liesbeth Couck, Hanna Tay, Fred Sinowatz, Wim Van Den Broeck	2024	Anatomia Histologia Embryologia	Scopus
10. Resistance mutations impair benzimidazole efficacy against <i>Ascaridia galli</i> by altering β-tubulin interactions	Jan Clyden B. Tenorio	2024	Microbes and Infectious Diseases	Scopus
11. Soil contamination with zoonotic geohelminths: emerging risks in the Philippines	Jan Clyden B. Tenorio	2024	Infectious Microbes & Diseases	Scopus
12. Zoonotic helminth infections in rodents and shrews from southern Philippines: Unforeseen One Health hazards	Mary Joyce H. Matullano, Jan Clyden B. Tenorio, Lothy F. Casim	2024	Veterinary Integrative Sciences	Scopus
13. Benzimidazole resistance-associated mutations improve the in-silico dimerization of hookworm tubulin: an additional resistance mechanism	Jan Clyden B. Tenorio, Muhammad Fikri Heikal, Alok Kafle, Prasert Saichua, Sutas Suttiaprapa	2024	Veterinary World	Scopus
14. Construction and validation of a novel multi-epitope in silico vaccine design against the paramyosin protein of <i>Opisthorchis viverrini</i> using immunoinformatics analyses	Alok Kafle, Jan Clyden B. Tenorio, Roshan Kumar Mahato, Sahara Dhakal, Muhammad F Heikal, Sutas Suttiaprapa	2024	Acta Tropica	Scopus

<p>15. Can Benzimidazole Resistance Undermine the Philippines' Success in Controlling and Eliminating Soil-Transmitted Helminth Infections? A Mini-Review</p>	<p>Jan Clyden B. Tenorio, Vicente Y Belizario Jr, Luis Fernando Viana Furtado, Sutas Suttiprapa</p>	<p>2024</p>	<p>Infectious Microbes & Diseases</p>	<p>Scopus</p>
<p>16. Benzimidazole resistance-associated mutations in the β-tubulin gene of hookworms: A systematic review</p>	<p>Jan Clyden B. Tenorio, Muhammad Fikri Heikal, Alok Kafle, Prasert Saichua, Sutas Suttiprapa</p>	<p>2024</p>	<p>Parasitology Research</p>	<p>Scopus</p>
<p>17. Epigenetic Biomarkers and the Wnt/β-Catenin Pathway in Opisthorchis viverrini-associated Cholangiocarcinoma: A Scoping Review on Therapeutic Opportunities</p>	<p>Alok Kafle, Sutas Suttiprapa, Mubarak Muhammad, Jan Clyden B. Tenorio, Roshan Kumar Mahato, Norhidayu Sahimin, Shih Keng Loong</p>	<p>2024</p>	<p>PLOS Neglected Tropical Diseases</p>	<p>Scopus</p>
<p>18. Ancylostoma ceylanicum and other zoonotic canine hookworms: neglected public and animal health risks in the Asia-Pacific region</p>	<p>Jan Clyden B. Tenorio, Ian Kim B Tabios, Tawin Inpankaew, Adrian P Ybañez, Saruda Tiwananthagorn, Sirikachorn Tangkawattana, Sutas Suttiprapa</p>	<p>2024</p>	<p>Animal Diseases</p>	<p>Scopus</p>
<p>19. Canine hookworms in the Philippines—Very common but very much neglected in veterinary research</p>	<p>Jan Clyden B. Tenorio</p>	<p>2023</p>	<p>Frontiers in Veterinary Science</p>	<p>Scopus</p>
<p>20. Ex-post impact analysis of the Philippine pig repopulation program</p>	<p>Lary Nel B Abao, Ruth M. Sonaco, Miguel V. Guioguo, Alvin Paul J. Dirain, Roland Y. Fajardo, Joseph Frank Mgayaya</p>	<p>2025</p>	<p>Evaluation and Program Planning</p>	<p>Scopus</p>
<p>21. Heat Stress: Can Animals be Stressed but still be Healthy?</p>	<p>Putri Kusuma Astuti, Roland Fajardo, George Wanjala, Nelly Kichamu, Husein Ohran, Bouabid Badaoui, Zoltán Bagi, Szilvia Kusza</p>	<p>2023/6/14</p>	<p>Scientific Papers Animal Science and Biotechnologies</p>	<p>Directory of Open Access Journals, EBSCO, CAB Abstracts, CABI Full Text, Index Copernicus, Ulrich's Periodicals Directory, Google Scholar, CiteFactor</p>

22. Continental vs. tropical breed: Immunity comparison under heat stress conditions utilizing qRT-PCR technique	Roland Y. Fajardo, Putri Kusuma Astuti, Zoltán Bagi, Lilla Bodrogi, Tímea Pintér, Gabriella Skoda, Husein Ohran, George Wanjala, Szilvia Kusza	2023	BIO Web of Conferences	Scopus
23. Heat stress: Can animals be stressed and still be healthy?	Putri Kusuma Astuti, Roland Y. Fajardo, George Wanjala, Nelly Kichamu, Husein Ohran, Bouabid Badaoui, Zoltán Bagi, Szilvia Kusza	2023/5/25	Multidisciplinary Conference on Sustainable Development	Scopus
24. Hungarian indigenous Tsigai, a promising breed for excellent heat tolerance and immunity	Putri Kusuma Astuti, Zoltán Bagi, Lilla Bodrogi, Tímea Pintér, Gabriella Skoda, Roland Y. Fajardo, Szilvia Kusza	2023/9/1	Saudi Journal of Biological Sciences	Scopus

Prepared by:

FLEURMINA EULA C. USOP

Department Research Coordinator, Department of Veterinary Technology

OPEN

Infectious Microbes & Diseases Publish Ahead of Print

DOI: 10.1097/IM9.0000000000000207

Effectiveness of Drug-Based Interventions and Integrated Community Strategies for Soil-Transmitted Helminth Control in Southeast Asia: A Systematic Review

Jan Clyden B. Tenorio^{1,2}, Alok Kufle¹, Muhammad Fikri Heikal¹, Prasert Saichua^{3,4}, Sutas Sutiprapa^{1,*}

Editor: Xiao-Nong Zhou

¹ Tropical Medicine Graduate Program, Department of Tropical Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand;

² Department of Veterinary Paraclinical Sciences, College of Veterinary Medicine, University of Southern Mindanao, Kabacan, Cotabato, Philippines;

³ Department of Tropical Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand;

⁴ WHO Collaborating Center for Research and Control of Opisthorchiasis (Southeast Asian Liver Fluke Disease), Tropical Disease Research Center, Khon Kaen University, Khon Kaen, Thailand.

***Corresponding author:** Sutas Sutiprapa, Department of Tropical Medicine, Faculty of Medicine, Khon Kaen University, 123 Moo 16 Muitsap Road, Khon Kaen 40002, Thailand. E-mail: sutasu@kku.ac.th

Author contributions: JCBT, PS and SS conceptualized this work; SS and PS supervised the research; JCBT, MFH and AK contributed to the methodology and formal analysis; JCBT wrote the original draft; MFH, AK, SS and PS reviewed the manuscript; all authors read and approved the manuscript.

Data availability: Due to the nature of this research, data were not generated. All information used for analysis is provided in this published article and its supplementary information files.

Funding: No funding was received for this work.

Conflicts of interest: The authors reported no conflicts of interest.

Copyright © 2026 the Author(s). Published by Wolters Kluwer Health, Inc. This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial License 4.0 (CCBY-NC), where it is permissible to download, share, remix, transform, and build upon the work provided it is properly cited. The work cannot be used commercially without permission from the journal.

Abstract

Soil-transmitted helminth (STH) infections continue to pose a significant health threat in Southeast Asia (SEA), where a third of global STH infections are estimated to occur. Preventive chemotherapy (PC) through mass treatments has been recommended by the WHO to combat STH infections and their dire consequences in susceptible populations. This review aims to update PC efforts for STH infection control in SEA. We systematically searched MEDLINE via PubMed, ScienceDirect, Scopus, EBSCO Host and ProQuest using specific terms for papers published from 2011 through 2025. From the 4294 initial search hits, 27 studies were included in this research. All SEA countries, except Brunei Darussalam and Singapore, were represented. Benzimidazole (BZ) drugs, namely, albendazole (400 mg) and mebendazole (500 mg), were the most commonly used drug interventions in PC efforts. BZ treatment effectively reduced the prevalence and intensity of STH infections, reinforcing its role in control programs. However, recent research has shown that BZ treatments fail to reduce infection prevalence and have diminished efficacy. Combination therapies, such as ivermectin (200 µg/kg) + albendazole (400 mg), enhanced treatment outcomes across STH species. Combination therapy integrated with health education and water, sanitation and hygiene improvements can accelerate control and elimination. Our review provides valuable insights for strengthening STH control programs in SEA, advocating for a multi-faceted approach informed by the current evidence.

Keywords: mass drug administration; neglected tropical disease; benzimidazoles; public health programs

Introduction

Soil-transmitted helminth (STH) infections remain a significant threat to public health in endemic areas with tropical and subtropical climates.¹ The WHO estimated that approximately 1.5 billion people are affected, accounting for approximately a quarter of the global population.² Recent data from the 2021 Global Burden of Disease study indicate that there are approximately 642 million cases worldwide, which contribute to 1.38 million disability-adjusted life-years.³ The age-standardized prevalence is 8429.89 per 100,000 population, representing a 70% decrease from the 1990 data.³ These infections are caused by *Ascaris lumbricoides*, *Trichuris trichiura*, hookworm species like *Necator americanus* and *Ancylostoma duodenale*, and *Strongyloides stercoralis*.⁴ *Ancylostoma ceylanicum* is a zoonotic hookworm that affects companion animals and humans in the Asia-Pacific region.⁵ Southeast Asia (SEA) is a hotspot for STH infections, accounting for one-third of all reported cases globally.⁶ A 2008 WHO report documented that the prevalence of STH infections exceeded 50% in some areas of Cambodia, Indonesia, Myanmar, the Philippines and Vietnam.⁷ A 2018 systematic review revealed that the prevalence of *A. lumbricoides* exceeded 20% in Myanmar, the Philippines, Malaysia and Indonesia, while hookworm infections surpassed this threshold in these countries and Cambodia.⁸ In addition, a recent analysis indicates that *T. trichiura* affects over 685 million people in SEA, with a pooled prevalence of 20.95%.⁹ The latest Philippine national survey revealed that the prevalence of STH infections was 8% higher than the 20% national target, and the prevalence of moderate-to-heavy infections more than 30% higher than the 2% target.^{10,11} Recent studies in Myanmar and Vietnam using qPCR and Kato-Katz smear, respectively, revealed alarmingly high rates of STH infections among schoolchildren, with 78.8% of this population infected in Myanmar (predominantly *A. lumbricoides* and *T. trichiura*) and 76.1% in Vietnam.^{12,13} In Malaysia and Thailand, persistently high STH infections in indigent and tribal communities remain a significant health problem, especially among children living in these remote and rural areas.^{14,15} STH infections have had a shared history with countries in SEA. Hence, it is not surprising that various efforts to control these parasitic health threats have been implemented across the region.

STH infections pose a significant health threat to vulnerable segments of the population within endemic regions, particularly schoolchildren, women of reproductive age and

immunocompromised individuals.¹⁴ Among children, the pathologic consequences of infection include iron-deficiency anemia, stunting, malnutrition, cognitive development issues and motor deficits.^{4,16,17} The severe effects of high-burden infections among schoolchildren have led to efforts to control, eliminate and prevent STH infections in this vulnerable population.¹⁴ The cornerstone of these efforts has been preventive chemotherapy (PC) with benzimidazole (BZ) drugs through mass drug administration (MDA) programs.^{18,19} Control and elimination efforts against STHs aim to reduce infection prevalence, lower the pathologic consequences of infections and prevent reinfection.^{16,20,21} According to 2023 WHO data, nearly 900 million preschool and school-aged children around the world require PC against STH infections.²² The 2018 WHO data showed that several endemic SEA countries had varying levels of PC coverage: Indonesia did not reach 75%, the Philippines and Vietnam achieved higher than 75%, but for less than five years, and only Cambodia, Lao PDR and Myanmar sustained a coverage above 75% for more than five years.²¹ Among preschool and school-aged children, the 2023 WHO data revealed that only Indonesia, Myanmar, Cambodia and Vietnam achieved the target 75% benchmark for PC coverage, while the Philippines and Lao PDR lagged behind, at 29.73% and 57.05%, respectively.²² SEA nations have demonstrated a sustained commitment to combating STH infections through various control programs and MDA initiatives. Significant disparities in coverage and long-term success highlight the ongoing challenges of achieving consistent and equitable elimination across the region. The fight against STH infections remains a difficult and ongoing process in endemic SEA countries. Trends and developments in these control and elimination efforts need to be assessed to forge the most appropriate path towards achieving national and global elimination targets.^{2,11,21} Hence, the objective of this comprehensive review is to provide an update on drug-based control and elimination efforts for the control and elimination of STH infections in SEA by summarizing and analyzing findings from peer-reviewed research. Here, we analyzed research published between 2011 and 2025 to document trends in PC using mono- and combination drug administration, present the value of integrated control efforts that include health education and water, sanitation and hygiene (WASH), and highlight research indicating diminished efficacy of MDA efforts from many countries in the region.

Results

Characteristics of the studies included

Five databases were searched, and a total of 4294 hits were retrieved (Figure 1). Of these, 67% were removed due to duplication. The initial screening of 1244 articles resulted in a 97% removal rate, with exclusions based on publication type (reviews and abstracts), study location (outside SEA), pathogen focus, publication date (outside January 2011 to January 2025), incomplete metadata (no authors/abstract), and language (non-English). Eligibility screening was conducted on the full text of the remaining 41 papers, among which 34% were excluded due to an incorrect study design or outcome. A total of 27 papers were included in the study (Table S1, <http://links.lww.com/IMD/A110>), and all of them passed the risk of bias assessment (Tables S2-4, <http://links.lww.com/IMD/A110>).

The included studies span all the countries of SEA, except Brunei Darussalam and Singapore, where PC is not required.²² Lao PDR had the greatest number of studies (six), followed by Myanmar, Indonesia and the Philippines (four). Three studies were conducted in Timor-Leste, while two studies each were performed in Thailand, Cambodia and Vietnam. Malaysia was represented by one study. Among these countries, Thailand and Malaysia do not require PC.²² Studies were performed in children (ie, preschool and school-aged) and community folks. Among the STHs studied, the most commonly represented was *T. trichiura*, followed by *A. lumbricoides* and hookworms. Most of the studies included in this review were analytical cross-sectional studies, followed by randomized controlled trials, with quasi-experimental studies being the least represented. Key information regarding the included studies is summarized in Tables 1–3.

BZ drugs, particularly albendazole (ABZ, 400 mg) and mebendazole (MBZ, 500 mg), were the most frequently employed interventions for MDA in the included studies. Combination therapy, in which drugs from different classes of anthelmintic are combined to expand the range of activity and increase efficacy, was also an emerging trend in the included studies. BZ drugs were combined with macrocyclic lactones such as ivermectin (IVM)³⁰ and pyrimidine derivatives such as pyrantel pamoate (PYR).²⁷ Similarly, a combination of drugs that integrate the control of numerous parasitic helminths of public health importance was also reported. For instance, the combination of ABZ, IVM and diethylcarbamazine (DEC) against lymphatic filariasis, scabies and STHs has

been tested for its ability to reduce infection prevalence.⁵⁶ Comprehensive control efforts that integrate drug-based interventions with health education, the provision of clean water, and the improvement of sanitation and hygiene standards have also been reported.^{26,33}

BZs as the primary intervention

Several cross-sectional studies included in this review assessed the effects of BZ monotherapy on infection prevalence. For instance, Sanza et al.³⁷ reported a significant decrease in the prevalence of STH infections in several provinces in the central Philippines where several rounds of MBZ monotherapy have been administered. Similarly, a preliminary study by Staal et al.⁴¹ reported a decrease in *A. lumbricoides*, *T. trichiura* and hookworm infection prevalence in Indonesia at follow-up after ABZ treatment. Studies on monotherapy and combination therapy have also reported the cure rate (CR) and egg reduction rate (ERR) as efficacy parameters. An early trial by Soukhathammavong et al. observed that ABZ and MBZ had subpar CR (less than 95%) against *A. lumbricoides*, but had superior ERR (almost 100%), while the efficacy against hookworms and *T. trichiura* was suboptimal, characterized by low CRs and ERRs.²⁸ A similar trend was observed by Levecke et al.²³ in trials conducted in Vietnam and Cambodia, and by Vaz Nery et al.⁴² among school-aged children in Timor Leste. A community-wide study by Ash et al. in the Lao PDR revealed that two rounds of triple-dose ABZ significantly reduced the prevalence and resulted in superior ERRs in *A. lumbricoides*, hookworms and *T. trichiura*.⁴⁹ In Vietnam, community-wide treatment with ABZ resulted in a more substantial reduction in the prevalence and intensity of infection than treatment involving only schoolchildren, as observed by Dyer et al.³¹ The use of BZ drugs for the control and elimination of STH infections relies on their efficacy, together with their safety and ease of administration.²¹ The challenge, however, is varied efficacies among different STH species.³¹ Hence, its combination with other dewormers from different classes has been explored.

Combination therapy: the new frontier?

Studies assessing the effects of combination therapy against STH infections have also reported varying outcomes. Sapulete et al.²³ assessed the efficacy of the combination of PYR and ABZ in comparison with ABZ alone in school children from Indonesia, which revealed that the addition of PYR increased the CR by 20%.²⁷ Another trial by Keller et al.³⁸ revealed that, compared with

ABZ monotherapy, IVM+ABZ combination treatment resulted in superior ERRs at 2 to 3 weeks, 6 months and 1 year after treatment. Utilizing qPCR as their diagnostic tool, Hürliemann et al.³² reported that, compared with ABZ monotherapy, the ABZ+IVM treatment resulted in higher CRs and ERRs for *A. lumbricoides*, *T. trichiura* and hookworms. The addition of DEC, IVM and ABZ resulted in a reduced infection prevalence of several hookworm species.³⁰

PC with health education and WASH initiatives: the ol' reliables

The combination of nonpharmaceutical interventions with drug-based treatments also showed promising effects on STH infections. The "Fit for School" program, which included daily handwashing with soap, daily brushing with fluoride toothpaste and biannual deworming with ABZ, resulted in a reduced prevalence of STH infections of moderate to heavy intensity.³³ Another innovation in health education was the incorporation of Magic Glasses, which is a health education intervention aimed at preventing intestinal worms in schoolchildren that involves using engaging cartoon videos and educational materials.⁵² The trial performed by Mationg et al.²⁹ in the Philippines revealed that the integration of the Magic Glasses program into the biannual ABZ administration program resulted in insignificant changes in the infection prevalence but markedly improved the knowledge and behavior of the schoolchildren receiving the intervention. In addition to health education, the provision of WASH infrastructure with deworming also improved infection control. A preliminary study showed that the provision of latrines and health education, together with ABZ treatment, significantly decreased the prevalence of STH infections among schoolchildren in Indonesia.⁵⁴ These results show the value of incorporating health education and WASH initiatives with drug administration for the control and elimination of STH infections. Recently, Mationg et al.⁵² conducted a review of studies that assessed the effectiveness of health education packages against STHs and their impacts on infection control. Only three relevant studies were identified, highlighting the neglect of this intervention in the current drug-based paradigm. As the articles included in our review purport, health education plays a crucial role in improving the knowledge and attitudes of the target populations. Likewise, improving WASH facilities enables the elimination of open defecation and improves hygiene practices that prevent STH infection and reinfection.³³ A multipronged approach that includes health education and

WASH facility provision with mass treatment can facilitate faster achievement of control and elimination targets.

Indications of diminished efficacy

Several alarming reports of reduced mass treatment efficacies, which were lower than the threshold in the WHO guidelines,⁵¹ were documented by some of the included studies. The WHO guideline stipulates that an anthelmintic drug's effectiveness is categorized as "satisfactory" if its ERR meets or exceeds the reference value, and as "doubtful" or "reduced" if the ERR falls below the reference value by less or more than 10 percentage points, respectively.⁵¹ The aforementioned study comparing the efficacy of ABZ and MBZ in Lao PDR reported doubtful ERR (ie, less than 10 percentage points lower than the 90% reference value) among hookworms when the former was administered.²⁴ More recently, Colella et al.⁴⁷ used qPCR to assess the efficacy of ABZ treatment and showed a diminished efficacy because ERRs of hookworm infections in Cambodia were reduced (ie, more than 10 percentage points lower than the 90% reference value). A similar trend was also observed in Timor-Leste, where ABZ treatment did not result in ERRs for *N. americanus* that were within satisfactory levels.⁴⁷ Persistent prevalence despite a history of repeated treatments was also reported. A history of BZ treatment in schoolchildren and women of reproductive age failed to decrease the prevalence of *A. lumbricoideis*, *T. trichiura* and hookworms in northern Lao PDR, where it remained relatively high.²⁶ In Myanmar, six rounds of ABZ treatment failed to consistently bring down the prevalence of *T. trichiura* infections.⁴⁸ The national and global control targets of the prevalence of STH infections have not been met after a decade of MDA with BZ drugs in schoolchildren in the Philippines, where the prevalence of *A. lumbricoideis* and *T. trichiura* remained higher than 50%.²⁸

Discussion

Our results show that BZ monotherapy remains central to STH control in Southeast Asia. However, some studies have reported that the efficacy of these drugs have been decreasing over time. To address this, combination therapy with ABZ+IVM has been trialed and has shown improved efficacy across different STH species. Moreover, health education regarding hygiene and improving access to clean water and sanitation in conjunction with drug administration have been shown to be effective and essential for the long-term control of STH infections. Based on the

current WHO roadmap, the primary goal for STH control is to achieve elimination as a public health problem, defined as reducing the prevalence of moderate-to-heavy intensity infections to below 1% in all endemic districts by 2030.⁵¹ The initial phase of intervention focuses on achieving disease control, which aims to significantly reduce the morbidity caused by these infections through regular MDA.¹⁸

Among the selected studies in this systematic review, BZ drugs, such as ABZ (400 mg) and MBZ (500 mg), were the most commonly used interventions in mass chemotherapy efforts. Indeed, MDA with BZ monotherapy remains the cornerstone of control and elimination efforts against STH infections.^{18,19} This is particularly true in other endemic regions such as South America,^{54,55} South Asia⁵⁶ and Africa.^{57,58} Several SEA nations recognized the serious threat of STH infections and established control programs early: Malaysia in 1974, Indonesia in 1975, Thailand in 1980 and the Philippines in 1999.⁸ Similarly, school-based deworming efforts were initiated in Vietnam, Lao PDR and Myanmar with aid from foreign agencies and support from affluent countries.⁸ Some of these programs were discontinued due to a lack of funding or reliance on donations, loans and support from foreign agencies for the drugs needed for mass PC.⁸ At the turn of the millennium, renewed enthusiasm for MDA against STH infections was seen. For instance, a 95% MDA coverage was reported during a country-wide, school-based deworming program conducted in Lao PDR in 2006.²⁸ Likewise, in 2006, the high infection prevalence reported previously prompted the Philippine government to implement the Integrated Helminth Control Program, which is still ongoing.^{7,11,19,60} Similar efforts were implemented in Indonesia through governmental programs and in Cambodia through foreign support.⁶¹

Similar to our results, reports from other regions affirm the enhanced efficacy when BZs are combined with IVM. A recent meta-analysis revealed a greater reduction in the prevalence of *T. trichiura* infections when ABZ+IVM was used than when IVM was used alone (89.40% vs. 49.93%).⁶² Against strongyloidiasis, however, IVM (84.49%) alone was slightly better in reducing infection prevalence than ABZ+IVM (81.37%).⁶² In India, ABZ+IVM was also shown to increase the ERR of *A. lumbricoideis* when compared with ABZ monotherapy (93.22% vs. 90.61%).⁶³ A cross-sectional study in a population receiving concurrent ABZ-IVM mass treatment in Cameroon revealed that the drug combination could have collateral impacts that can hasten the control and

elimination of STHs.⁶⁴ Moreover, the efficacy of ABZ+IVM in reducing the incidence of STH-associated anemia and infections and their relative safety during pregnancy, were assessed in Uganda.⁶⁵ The results of the studies included in our review and those from the literature indicate that adding drugs from different classes to BZs improved the efficacy of treatments across a variety of STH species that affect populations within endemic areas. This point was highlighted by Emerson et al.,⁶⁶ which reported that combination therapy with appropriate drugs targeted toward the most susceptible helminth species can hasten the achievement of global control and elimination targets and therefore lessen the risk of resistance development.

The results from the selected papers indicate that mass treatments with BZs may have reduced efficacy depending on the species of the helminth and the drug used. This issue provides an opportunity for combination therapy regimens that can enhance efficacy, as proven by the aforementioned studies. The long-term consequences of combination treatment against STHs have yet to be fully assessed. However, the emergence of multidrug-resistant STHs as a consequence of combination therapy has become a concern.⁶⁷⁻⁶⁹ Likewise, evidence of multidrug-resistant animal hookworms due to the protracted use of combination therapy in the United States and Canada should also be kept in mind.¹⁰⁻¹² The role of other factors, such as treatment coverage and compliance, needs to be thoroughly evaluated in all endemic countries in SEA. Moreover, the indication of resistance has yet to be fully elucidated by field data in this region. Guidelines on the determination of resistance in STHs have yet to be promulgated.⁷³ Only guidelines for the assessment of PC efficacy against STH infections and schistosomiasis have been established by the WHO.⁵¹

Our comprehensive review has several limitations. First, we were not able to incorporate data from governmental databases because we focused only on peer-reviewed literature. Some PC efforts may not be described in peer-reviewed literature. However, through extensive searches and sufficient selection and eligibility criteria, we were able to include studies that were performed in areas where government-implemented MDA was being carried out, such as those in the Philippines^{21,26} and Lao PDR.²⁶ Second, the variability in the sample sizes, control groups used (if necessary), outcomes measured and study designs hamper the integrated analysis of the data from the included studies. Key limitations arise from the necessity to acknowledge the potential

publication and language bias inherent in restricting the search to nonmultilingual and indexed sources. The methodological rigor of the overall synthesis is also limited by the inadequate statistical power of many individual trials due to their small sample sizes. A final constraint is the absence of longitudinal data beyond five years, making it impossible to evaluate long-term effectiveness or the definitive emergence of resistance. Despite these issues, our utilization of appropriate risk-of-bias tools allowed for the assessment of each study's quality, confirming that the included results and their synthesis were of acceptable scientific merit.

Conclusions

This comprehensive review aimed to update the knowledge on mass treatment for STH control in SEA countries by collecting and synthesizing peer-reviewed literature. We searched several databases and included 27 studies that passed our screening and eligibility criteria. These papers had different study designs and utilized various drug interventions. BZ drugs, primarily ABZ and MBZ, remained the most commonly used anthelmintic in mass deworming efforts in the region, with often satisfactory but inconsistent results in terms of efficacy and reducing infection prevalence. An emerging trend that the papers reported on is the combination of anthelmintics from different classes with BZs to enhance efficacy. Moreover, comprehensive efforts that include health education and WASH facility improvement with deworming result in enhanced infection reduction. Several reports have documented diminished efficacies, such as insufficient ERRs and failure to significantly reduce infection prevalence, when BZs are used against hookworms and *T. trichiura* infections. Combination therapy (eg, ABZ+IVM) provides an opportunity to address this issue and enhance efficacy. Therefore, judicious combination therapy with health education packages and improvement of WASH can hasten the achievement of control and elimination targets and abate the threat of resistance.

Materials and methods

Research questions

This systematic review aimed to answer the following research questions:

1. Which SEA countries have implemented drug-based STH control efforts, as reported in peer-reviewed literature published from 2011 through 2025?
2. What drugs have been used as interventions in these control efforts?

3. What were the treatment outcomes of drug administration in controlling infections in these countries?

Search strategy

Five databases were searched in this systematic review, MEDLINE via PubMed (<https://pubmed.ncbi.nlm.nih.gov/>), Scopus (<https://www.scopus.com/sources>), Science Direct (<https://www.sciencedirect.com/search/entry>), EBSCO Host (<https://search.ebscohost.com/>) and ProQuest (<https://www.proquest.com/>) were searched for literature (accessed 7 through 11 February 2025). All database searches were restricted to the publication date from January 2011 through January 2025. This time restriction was chosen to provide a follow-up to the review on the same topic by Jex et al.⁴ This review is limited to the member countries of the Association of Southeast Asian Nations. For EBSCO Host, the Applied Science and Technology Source Ultimate, CINAHL Complete, MEDLINE and News wires databases were selected. The specific terms used to search the databases can be found in Table S5, <http://links.lww.com/IMD/A110>. The .ris and .nbib files of the searches were downloaded.

Study screening, selection and eligibility

The search results were uploaded to Rayyan (<https://www.rayyan.ai/>), where study selection, screening and eligibility checking were performed (accessed 14 through 21 February 2025). Rayyan is an AI-assisted platform for streamlining study screening, with the ability to import and deduplicate references from various sources.¹⁴ The platform was used for duplicate detection, with 95% text similarity as the resolution limit. Duplicates with <95% similarity were resolved manually. Titles and abstracts were used for the initial screening, and papers lacking these components were excluded. The detailed inclusion and exclusion criteria used for assessment are summarized in Table S6, <http://links.lww.com/IMD/A110>. The first, second and third authors conducted the study selection, screening and eligibility assessment, and all authors collaboratively resolved disagreements to reach a consensus. An overview of the study selection, screening and eligibility assessment process is provided in Figure 1.

Risk of bias assessment, data extraction and synthesis

The papers finally included in this study were of several types: cross-sectional studies, quasi-experimental studies and randomized controlled trials. Due to the diversity of study types, several

risk-of-bias tools were utilized. The *Joanna Briggs Institute's Critical Appraisal Checklist for Analytical Cross-Sectional Studies*, the *Critical Appraisal Checklist for Quasi-Experimental Studies*, and the *Critical Appraisal Tool for Assessment of Risk of Bias for Randomized Controlled Trials* were used (<https://jbi.global/critical-appraisal-tools>). The second and third authors independently reviewed the risk of bias assessment. The fourth and fifth authors resolved disagreements. The results of the risk of bias assessment are provided in Tables S1-3, <http://links.lww.com/IMD/A110>.

Codes were assigned to the included papers (Table S6, <http://links.lww.com/IMD/A110>). Data extracted from the studies include authors' names, year of publication, title, type of study, specific locale and country of the study, population, STH species studied, drug-based intervention, comparison groups (if any), test used and key outcomes. These were input into a spreadsheet. The study results were described, summarized and synthesized using descriptive statistics, specifically counts and percentages.

Supplementary digital contents

Supplementary materials are available at, <http://links.lww.com/IMD/A110>

Acknowledgments

JCBT and AK would like to acknowledge the Postgraduate Scholarship for International Students of the Faculty of Medicine, Khon Kaen University for funding their doctoral studies. Additionally, MFH acknowledges Khon Kaen University Active Recruitment Scholarships for funding his master's degree studies. SS is supported by the Fundamental Fund of Khon Kaen University and the National Science, Research, and Innovation Fund.

References

1. Lebu S, Kibone W, Muoghalu CC, et al. Soil-transmitted helminths: a critical review of the impact of co-infections and implications for control and elimination. *PLoS Negl Trop Dis* 2023;17(8):e0011496. <https://doi.org/10.1371/journal.pntd.0011496>
2. *Soil-transmitted Helminth Infections*. World Health Organization. Available at: <https://www.who.int/news-room/fact-sheets/detail/soil-transmitted-helminth-infections>. Accessed September 16, 2023.
3. Chen J, Gong Y, Chen Q, Li S, Zhou Y. Global burden of soil-transmitted helminth infections, 1990–2021. *Infect Dis Poverty* 2024;13(1):77. <https://doi.org/10.1186/s40249-024-01238-9>
4. Jourdan PM, Lamberton PHL, Fenwick A, Addiss DG. Soil-transmitted helminth infections. *Lancet* 2018;391(10117):252–265. [https://doi.org/10.1016/S0140-6736\(17\)31930-X](https://doi.org/10.1016/S0140-6736(17)31930-X)
5. Tenorio JCB, Tabios IKB, Inpankaew T, et al. *Ancylostoma ceylanicum* and other zoonotic canine hookworms: neglected public and animal health risks in the Asia–Pacific region. *Anim Dis* 2024;4(1):11. <https://doi.org/10.1186/s44149-024-00117-y>
6. Jex AR, Lim YAL, Bethony JM, Hotez PJ, Young ND, Gasser RB. Soil-transmitted helminths of humans in Southeast Asia—towards integrated control. In: Rollinson D, Hay SI, eds. *Advances in Parasitology*. Vol 74. Amsterdam: Elsevier;2011:231–265. <https://doi.org/10.1016/b978-0-12-385897-9.00004-5>
7. *Review on the Epidemiological Profile of Helminthiases and their Control in the Western Pacific Region, 1997–2008*. World Health Organization. Published online 2008. <https://www.who.int/publications/item/WHO-MVP-WPRO-2008.1>. February 26, 2025.
8. Silver ZA, Kaliappan SP, Samuel P, et al. Geographical distribution of soil transmitted helminths and the effects of community type in South Asia and South East Asia - a systematic review. *PLoS Negl Trop Dis* 2018;12(1):e0006153. <https://doi.org/10.1371/journal.pntd.0006153>
9. Behniafar H, Sepidarkish M, Tadi MJ, et al. The global prevalence of *Trichuris trichiura* infection in humans (2010–2023): a systematic review and meta-analysis. *J Infect Public Health* 2024;17(5):800–809. <https://doi.org/10.1016/j.jiph.2024.03.005>
10. Tangcalagan D, Daga C, Tan A, et al. The 2013–2015 nationwide prevalence survey of soil-transmitted helminths (STH) and schistosomiasis among school-age children in public schools in the Philippines. *Pediatr Infect Dis Soc Philipp J* 2022;23(1):75–96. <https://doi.org/10.56964/pidspj20222301010>
11. Tenorio JCB, Belizario VY, Furtado LFV, Suttiprapa S. Can benzimidazole resistance undermine the Philippines' success in controlling and eliminating soil-transmitted helminth infections? A mini-review. *Infect Microb Dis* 2024;6(4):163–169. <https://doi.org/10.1097/im9.000000000000163>
12. Aung E, Han KT, Gordon CA, et al. High prevalence of soil-transmitted helminth infections in Myanmar schoolchildren. *Infect Dis Poverty* 2022;11(1):28. <https://doi.org/10.1186/s40249-022-00952-6>
13. Nhu DD, Ly TT, Canh HD, et al. High prevalence of soil-transmitted helminths among primary students in Ha Giang, Vietnam: a descriptive cross-sectional study. *Medicine* 2025;104(3):e41120. <https://doi.org/10.1097/MD.00000000000041120>
14. Mohammad Z, Ibrahim AA, Reffien MAM, et al. Prevalence of soil-transmitted helminth infections in Malaysia: a systematic review and meta-analysis. *Open Access Maced J Med Sci* 2024;12(1):48–55. <https://doi.org/10.3889/oamjms.2024.11467>
15. Wisetmora A, Wattanawong O, Wiji A, et al. Gastrointestinal helminthic infection among the population in northern Thailand. *Acta Parasitol* 2024;69(3):1648–1660. <https://doi.org/10.1007/s11686-024-00892-1>
16. Owada K, Nielsen M, Lau CL, Clements ACA, Yakob L, Soares Magalhães RJ. Measuring the effect of soil-transmitted helminth infections on cognitive function in children. In: Rollinson D, Stothard JR, eds. *Advances in Parasitology*. Vol 98. Amsterdam: Elsevier; 2017:1–37. <https://doi.org/10.1016/b978-0-12-385897-9.00004-5>
17. Owada K, Nielsen M, Lau CL, et al. Functional illiteracy burden in soil-transmitted helminth (STH) endemic regions of the Philippines: an ecological study and geographical prediction for 2017. *PLoS Negl Trop Dis* 2019;13(6):e0007494. <https://doi.org/10.1371/journal.pntd.0007494>

18. *Guideline: Preventive Chemotherapy to Control Soil-Transmitted Helminth Infections in at-Risk Population Groups*. World Health Organization. Available at: <https://apps.who.int/iris/handle/10665/255983>. Accessed August 24, 2023.
19. Maciong MLS, Tallo VL, Williams GM, et al. The control of soil-transmitted helminthiases in the Philippines: the story continues. *Infect Dis Poverty* 2021;10(1):85. <https://doi.org/10.1186/s40249-021-00870-z>
20. Anderson R, Hollingsworth TD, Traacott J, Brooker S. Optimisation of mass chemotherapy to control soil-transmitted helminth infection. *Lancet* 2012;379(9813):289–290. [https://doi.org/10.1016/S0140-6736\(12\)60120-2](https://doi.org/10.1016/S0140-6736(12)60120-2)
21. Montresor A, Mupfasoni D, Mikhailov A, et al. The global progress of soil-transmitted helminthiases control in 2020 and World Health Organization targets for 2030. *PLoS Negl Trop Dis* 2020;14(8): e0008505. <https://doi.org/10.1371/journal.pntd.0008505>
22. *Soil-transmitted helminthiases Interactive Graph (Global Health Observatory)*. World Health Organization. https://apps.who.int/neglected_diseases/middata/sth/sth.html. Accessed February 26, 2025.
23. *Ending the Neglect to Attain the Sustainable Development Goals A Road Map for Neglected Tropical Diseases 2021–2030*. World Health Organization. <https://www.who.int/publications/i/item/9789240010352>. Accessed February 26, 2025.
24. Soukhathammavong PA, Sayasone S, Phongluxa K, et al. Low efficacy of single-dose albendazole and mebendazole against hookworm and effect on concomitant helminth infection in Lao PDR. *PLoS Negl Trop Dis* 2012;6(1):e1417. <https://doi.org/10.1371/journal.pntd.0001417>
25. Levecke B, Montresor A, Albonico M, et al. Assessment of anthelmintic efficacy of mebendazole in school children in six countries where soil-transmitted helminths are endemic. *PLoS Negl Trop Dis* 2014;8(10):e3204. <https://doi.org/10.1371/journal.pntd.0003204>
26. Clarke NE, Clements ACA, Amaral S, et al. (S)WASH-D for Worms: a pilot study investigating the differential impact of school- versus community-based integrated control programs for soil-transmitted helminths. *PLoS Negl Trop Dis* 2018;12(5):e0006389. <https://doi.org/10.1371/journal.pntd.0006389>
27. Sapulete EJJ, de Dwi Lingga Utama IMG, Sanjaya Putra IGN, Kanya Wati D, Arimbawa IM, Gustawan IW. Efficacy of albendazole-pyrantel pamoate compared to albendazole alone for *Trichuris trichiura* infection in children: a double blind randomised controlled trial. *Malays J Med Sci* 2020;27(3):67–74. <https://doi.org/10.21315/mjms2020.27.3.7>
28. Keller L, Welsche S, Patel C, et al. Long-term outcomes of ivermectin-albendazole versus albendazole alone against soil-transmitted helminths: Results from randomized controlled trials in Lao PDR and Pemba Island, Tanzania. *PLoS Negl Trop Dis* 2021;15(6):e0009561. <https://doi.org/10.1371/journal.pntd.0009561>
29. Maciong MLS, Williams GM, Tallo VL, et al. “The Magic Glasses Philippines”: a cluster randomised controlled trial of a health education package for the prevention of intestinal worm infections in schoolchildren. *Lancet Reg Health West Pac* 2022;18:100312. <https://doi.org/10.1016/j.lanwpc.2021.100312>
30. Hürlimann E, Keller L, Patel C, et al. Efficacy and safety of co-administered ivermectin and albendazole in school-aged children and adults infected with *Trichuris trichiura* in Côte d’Ivoire, Laos, and Pemba Island, Tanzania: a double-blind, parallel-group, phase 3, randomised controlled trial. *Lancet Infect Dis* 2022;22(1):123–135. [https://doi.org/10.1016/S1473-3099\(21\)00421-7](https://doi.org/10.1016/S1473-3099(21)00421-7)
31. Dyer CEP, Ng-Nguyen D, Clarke NE, et al. Community-wide versus school-based targeted deworming for soil-transmitted helminth control in school-aged children in Vietnam: the CoDe-STH cluster-randomised controlled trial. *Lancet Reg Health West Pac* 2023;4(1):100920. <https://doi.org/10.1016/j.lanwpc.2023.100920>
32. Welsche S, Schmeberger PHH, Hattendorf J, Sayasone S, Hürlimann E, Keiser J. Egg excretion patterns of soil-transmitted helminth infections in humans following albendazole-ivermectin and albendazole treatment. *PLoS Negl Trop Dis* 2024;18(3):e0012073. <https://doi.org/10.1371/journal.pntd.0012073>
33. Monse B, Benziou H, Naliponguit E, Belizario V, Schratz A, van Palenstein Heiderman W. The Fit for School Health Outcome Study - a longitudinal survey to assess health impacts of

- an integrated school health programme in the Philippines. *BMC Public Health* 2013;13:256. <https://doi.org/10.1186/1471-2458-13-256>
34. Park MJ, Laaksono B, Clements A, Sadler R, Stewart D. Worm-free children: an integrated approach to reduction of soil-transmitted helminth infections in Central Java. *Rev Environ Health* 2016;31(1):111–113. <https://doi.org/10.1515/revel-2015-0053>
 35. Nachaiwieng W, Sanit S, Kongsa N, et al. The impact of an integrated intervention program combining drug therapy with water, sanitation, and hygiene (WASH) education on reinfection with intestinal parasitic infections among the Karen hill tribe in northern Thailand. *Parasit Vectors* 2024;17(1):544. <https://doi.org/10.1186/s13071-024-06611-z>
 36. Conlan JV, Khamlome B, Vongxay K, et al. Soil-transmitted helminthiasis in Laos: a community-wide cross-sectional study of humans and dogs in a mass drug administration environment. *Am J Trop Med Hyg* 2012;86(4):624–634. <https://doi.org/10.4269/ajtmh.2012.11-0413>
 37. Sanza M, Totanes FI, Chua PL, Belizario VY. Monitoring the impact of a mebendazole mass drug administration initiative for soil-transmitted helminthiasis (STH) control in the Western Visayas Region of the Philippines from 2007 through 2011. *Acta Trop* 2013;127(2):112–117. <https://doi.org/10.1016/j.actatropica.2013.03.017>
 38. Belizario VY, Ng JV, Amacillo MLE, Deles Trizos JPC, Reyes M, Fudalen O. High burden of soil-transmitted helminthiasis in preschool-age children in Masbate: a decade of implementation of the Integrated Helminth Control Program in the Philippines. *Southeast Asian J Trop Med Public Health*. 2016;47(4):667–679.
 39. Dunn JC, Bettis AA, Wyine NY, et al. A cross-sectional survey of soil-transmitted helminthiasis in two Myanmar villages receiving mass drug administration: epidemiology of infection with a focus on adults. *Parasit Vectors* 2017;10(1):374. <https://doi.org/10.1186/s13071-017-2306-2>
 40. Ash A, Okello A, Khamlome B, Inthavong P, Allen J, Thompson RCA. Controlling *Taenia solium* and soil transmitted helminths in a northern Lao PDR village: Impact of a triple dose albendazole regime. *Acta Trop* 2017;174:171–178. <https://doi.org/10.1016/j.actatropica.2015.05.018>
 41. Staal SL, Hogendoorn SKI, Voets SA, et al. Prevalence of atopy following mass drug administration with albendazole: a study in school children on Flores Island, Indonesia. *Int Arch Allergy Immunol* 2018;177(3):192–198. <https://doi.org/10.1159/000490952>
 42. Vaz Nery S, Qi J, Llewellyn S, et al. Use of quantitative PCR to assess the efficacy of albendazole against *Necator americanus* and *Ascaris* spp. in Manufahi District, Timor-Leste. *Parasit Vectors* 2018;11(1):373. <https://doi.org/10.1186/s13071-018-2838-0>
 43. Sungkar S, Tambunan FBJ, Gozali MN, Kusumowidagdo G, Wahdini S. The effect of albendazole toward *Anemia* in children with soil-transmitted helminths infection in a remote and endemic area. *Med J Indones* 2018;27(4):293-298. <https://doi.org/10.13181/mji.v27i4.2857>
 44. Dunn JC, Bettis AA, Wyine NY, et al. Soil-transmitted helminth reinfection four and six months after mass drug administration: results from the delta region of Myanmar. *PLoS Negl Trop Dis* 2019;13(2):e0006591. <https://doi.org/10.1371/journal.pntd.0006591>
 45. Chai JY, Sohn WM, Hong SJ, et al. Effect of mass drug administration with a single dose of albendazole on *Ascaris lumbricoides* and *Trichuris trichiura* infection among schoolchildren in Yangon Region, Myanmar. *Korean J Parasitol* 2020;58(2):195–200. <https://doi.org/10.3347/kjp.2020.58.2.195>
 46. Stracke K, Adisakwattana P, Phuanakoonnon S, et al. Field evaluation of the gut microbiome composition of pre-school and school-aged children in Tha Song Yang, Thailand, following oral MDA for STH infections. *PLoS Negl Trop Dis* 2021;15(7):e0009597. <https://doi.org/10.1371/journal.pntd.0009597>
 47. Colella V, Khieu V, Worsley A, et al. Risk profiling and efficacy of albendazole against the hookworms *Necator americanus* and *Ancylostoma ceylanicum* in Cambodia to support control programs in Southeast Asia and the Western Pacific. *Lancet Reg Health West Pac* 2021;16:100258. <https://doi.org/10.1016/j.lanwpc.2021.100258>
 48. Hong S, Jung BK, Song H, et al. Failure of repeated MDA with albendazole for trichuriasis control in schoolchildren of the Yangon Region, Myanmar. *Korean J Parasitol* 2021;59(6):607–613. <https://doi.org/10.3347/kjp.2021.59.6.607>

49. Tee MZ, Lee SC, Fr YX, et al. Efficacy of triple dose albendazole treatment for soil-transmitted helminth infections. *PLoS One* 2022;17(8):e0272821. <https://doi.org/10.1371/journal.pone.0272821>
50. Le B, Monteiro MAA, Ansari S, et al. The impact of ivermectin, diethylcarbamazine citrate, and albendazole mass drug administration on the prevalence of Scabies and soil-transmitted helminths in school-aged children in three municipalities in Timor-Leste: a before-after assessment. *Lancet Glob Health* 2023;11(6):e924-e932. [https://doi.org/10.1016/S2214-109X\(23\)00134-1](https://doi.org/10.1016/S2214-109X(23)00134-1)
51. *Assessing the Efficacy of Anthelmintic Drugs against Schistosomiasis and Soil-Transmitted Helminthiasis*. World Health Organization. Available at: <https://apps.who.int/iris/handle/10665/79019>. Accessed August 20, 2023.
52. Matiang MLS, Williams GM, Tallo VL, et al. A review of health education activities targeting schoolchildren for the control of soil-transmitted helminthiasis in Southeast Asia, with emphasis upon the Magic Glasses approach. (In: Rollinson D, Stothard R, eds. *Advances in Parasitology*. Vol 123. Advances in Parasitology Amsterdam: Elsevier, 2024:1-22. <https://doi.org/10.1016/bs.apar.2023.12.001>
53. Vaz Nery S, Pickering AJ, Abate E, et al. The role of water, sanitation and hygiene interventions in reducing soil-transmitted helminths: interpreting the evidence and identifying next steps. *Parasit Vectors* 2019;12(1):273. <https://doi.org/10.1186/s13071-019-3532-6>
54. Servino A, Garimano N, Santini MS. Systematic review and meta-analysis of soil-transmitted helminth infections in South America (2000-2024). *Acta Trop* 2024;260:107400. <https://doi.org/10.1016/j.actatropica.2024.107400>
55. Cabada MM, Lopez M, Asque E, Clinton White A. Prevalence of soil-transmitted helminths after mass albendazole administration in an indigenous community of the Manu jungle in Peru. *Pathog Glob Health* 2014;108(4):200-205. <https://doi.org/10.1179/2047773214Y.0000000142>
56. Abraham D, Kaliappan SP, Watson JL, Rao Ajjampur SS. Intervention strategies to reduce the burden of soil-transmitted helminths in India. *Indian J Med Res* 2018;147(6):533-544. https://doi.org/10.4103/ijmr.UMR_881_18
57. Sissy M, Damie D, Hailu T. Efficacy of albendazole against soil-transmitted helminth infections in Ethiopia: a systematic review and meta-analysis. *Sci Rep* 2024;14(1):21970. <https://doi.org/10.1038/s41598-024-71308-3>
58. Kabotende J, Barry A, Mugisha M, et al. Efficacy of single-dose albendazole for the treatment of soil-transmitted helminthic infections among school children in Rwanda—a prospective cohort study. *Pharmaceuticals* 2023;16(2):139. <https://doi.org/10.3390/ph16020139>
59. Phommassack B, Sakloekham K, Chanthavisouk C, et al. Coverage and costs of a school deworming programme in 2007 targeting all primary schools in Lao PDR. *Trans R Soc Trop Med Hyg* 2008;102(12):1201-1206. <https://doi.org/10.1016/j.trstmh.2008.04.036>
60. *Administrative Order 2006-0028: Strategic and Operational Framework for Establishing Integrated Helminth Control Program*. Philippine Department of Health. https://doh.gov.ph/sites/default/files/health_programs/IHCP%20Operational%20and%20Strategic%20framework%20-%20AO%2006-0028.pdf. Accessed February 26, 2025.
61. Crompton DWT, Montresor A, Nsoamenang MC, Savioli L. *Controlling Disease Due to Helminth Infections*. World Health Organization. available at: <https://iris.who.int/server/api/core/bitstreams/213d9f0e-8c6a-41fe-a2a3-bbd82f08f7bd/content>. Accessed February 26, 2025.
62. Le B, Clarke NE, Legrand N, Nery SV. Effectiveness of ivermectin mass drug administration in the control of soil-transmitted helminth infections in endemic populations: a systematic review and meta-analysis. *Infect Dis Poverty* 2024;13(1):16. <https://doi.org/10.1186/s40249-024-01185-5>
63. Awasthi S, Nandy M, Datta K, et al. Efficacy and safety of albendazole versus albendazole and ivermectin in reduction of soil-transmitted helminth infections in school-age children (DVERMIN): a double-blind, randomized, controlled trial. *Cureus* 2025;17(2):e79152. <https://doi.org/10.7759/cureus.79152>

64. Djime-Yemeli I, Nana-Djeunga HC, Ikenou-Nanga CG, et al. Serious limitations of the current strategy to control soil-transmitted helminths and added value of ivermectin/albendazole mass administration: a population-based observational study in Cameroon. *PLoS Negl Trop Dis* 2020;14(11):e0008794. <https://doi.org/10.1371/journal.pntd.0008794>

65. Ndyomugenyi R, Kabstereine N, Olsen A, Magnussen P. Efficacy of ivermectin and albendazole alone and in combination for treatment of soil-transmitted helminths in pregnancy and adverse events: a randomized open label controlled intervention trial in Masindi district, western Uganda. *Am J Trop Med Hyg* 2008;79(6):856–863.

66. Emerson PM, Evans D, Freeman MC, et al. Need for a paradigm shift in soil-transmitted helminthiasis control: targeting the right people, in the right place, and with the right drug(s). *PLoS Negl Trop Dis* 2024;18(10):e0012521. <https://doi.org/10.1371/journal.pntd.0012521>

67. Ng'etich AI, Amoah ID, Bux F, Kumari S. Anthelmintic resistance in soil-transmitted helminths: One-Health considerations. *Parasitol Res* 2023;123(1):62. <https://doi.org/10.1007/s00436-023-08088-8>

68. Tenorio JC. Drug resistance in parasites: a review of mechanisms, drivers, and mitigation strategies. *Microbes Infect Dis* 2025;6(3):3. <https://doi.org/10.21608/mid.2024.317774.2189>

69. Tinkler SH. Preventive chemotherapy and anthelmintic resistance of soil-transmitted helminths - can we learn nothing from veterinary medicine? *One Health* 2019;9:100106. <https://doi.org/10.1016/j.onehit.2019.100106>

70. Jiménez-Castro PD, Howell SB, Schaefer JJ, Avramenko RW, Gilleard JS, Kaplan RM. Multiple drug resistance in the canine hookworm *Ancylostoma caninum*: an emerging threat? *Parasit Vectors* 2019;12(1):576. <https://doi.org/10.1186/s13071-019-3828-6>

71. Tenorio JCB, Heikal MF, Kalle A, Saichua P, Suttiaprapa S. Benzimidazole resistance-associated mutations in the β -tubulin gene of hookworms: a systematic review. *Parasitol Res* 2024;123(12):405. <https://doi.org/10.1007/s00436-024-08432-6>

72. Venkatesan A, Jimenez-Castro PD, Morosetti A, et al. Molecular evidence of widespread benzimidazole drug resistance in *Ancylostoma caninum* from domestic dogs throughout the

USA and discovery of a novel β -tubulin benzimidazole resistance mutation. *PLoS Pathog* 2023;19(3):e1011146. <https://doi.org/10.1371/journal.ppat.1011146>

73. Freeman MC, Akogun O, Belizario V Jr, et al. Challenges and opportunities for control and elimination of soil-transmitted helminth infection beyond 2020. *PLoS Negl Trop Dis* 2019;13(4):e0007201. <https://doi.org/10.1371/journal.pntd.0007201>

74. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan-a web and mobile app for systematic reviews. *Syst Rev* 2016;5(1):210. <https://doi.org/10.1186/s13643-016-0384-4>

Figure legends

Figure 1. PRISMA summary of the systematic literature search, screening and selection process of this review. STH, soil-transmitted helminth.

Table 1. Key results from randomized controlled trials included in this review

Study	Country	Location	Population	STH studied	MDA intervention (dose)	Comparison group	Test used	Key outcomes
Soukthasomnavong et al. (2012) ²⁶	Laos	Batsong district, Champasak Province	Children aged 6–12 y	Hookworms, <i>Ascariis</i> , <i>Trichuris</i>	ABZ (400 mg)	MBZ (500 mg)	Kato-Katz thick smear	Hookworms: ABZ CR, 32.0%; ABZ ERR, 86.7%; MBZ CR, 15%; MBZ ERR, 76.3% <i>Ascaris</i> : ABZ CR, 92.9%; ABZ ERR, 100%; MBZ CR, 93.3%; MBZ ERR, 100% <i>Trichuris</i> : ABZ CR, 33.3%; ABZ ERR, 67%; MBZ CR, 27.9%; MBZ ERR, 66%

Study	Country	Location	Population	STH studied	MDA intervention (dose)	Comparison group	Test used	Key outcomes
Levecke et al. (2014) ²²	Cambodia and Vietnam	Cambodia and Vietnam	School children aged 4-18 y	Hookworms, <i>Ascariis</i> , <i>Trichuris</i>	MBZ (500 mg)	ABZ (400 mg)	McMaster egg counting method	<p><i>Ascariis</i>: ABZ, 100%; FECC in Vietnam, 93.9%; FECC in Vietnam</p> <p><i>Trichuris</i>: ABZ, 92.3%; FECC in Vietnam; MBZ, 76.8%; FECC in Vietnam</p> <p>Hookworms: ABZ, 97.6%; FECC in Cambodia; MBZ, 79.7%; FECC in Cambodia; ABZ, 100%; FECC in Vietnam; MBZ, 95%; FECC in Vietnam</p>
Clarke et al. (2018) ¹⁴	Timor-Leste	Aileu and Manufohi municipalities	Community-wide and school children	Hookworms, <i>Ascariis</i> , <i>Trichuris</i>	Community-wide ABZ (400 mg) MDA and WASH only in schools	ABZ (400 mg) MDA and WASH only in schools	Real-time multiplex PCR	<p>Control arm prevalence: <i>Ascariis</i>, 48.7%; <i>Trichuris</i>, 2.2%; <i>Necator americanus</i>, 13.7%</p> <p>Intervention arm prevalence: <i>Ascariis</i>, 7.6%; <i>Trichuris</i>, 1.9%; <i>N. americanus</i>, 15.1%</p>

Study	Country	Location	Population	STH studied	MDA intervention (dose)	Comparison group	Test used	Key outcomes
Sapulete et al. (2020) ²³	Indonesia	Bangli, Bali	Children aged 8-12 y	<i>Trichuris</i>	ABZ (400 mg) and PYR (103 mg/kg) for 3 consecutive d	ABZ (400 mg) for 3 consecutive d	Kato-Katz thick smear	<p>Control arm CR, 40%; intervention arm CR, 60%</p> <p>Control arm % satisfactory ERR (≥ 50%), 44.7%; intervention arm % satisfactory ERR (≥ 50%), 55.3%</p>
Keller et al. (2021) ²⁴	Lao PDR	Nam Bak district in Luang Prabang Province	Community-wide, aged 6-60 y	Hookworms, <i>Ascariis</i> , <i>Trichuris</i>	ABZ (400 mg) and IVM (200 µg/kg)	ABZ (400 mg)	Kato-Katz thick smear	<p>14- to 21-d ERR: control arm, 68.8%; intervention arm, 99.2%</p> <p>6-mo ERR: control arm, 79.6%; intervention arm, 99%</p> <p>12-mo ERR: control arm, 91.3%; intervention arm, 99.0%</p>

Study	Country	Location	Population	STH studied	MDA intervention (dose)	Comparison group	Test used	Key outcomes
Maitong et al. (2021) ²⁹	Philippines	Laguna Province	School children aged 9-10 y	Total STHs	ABZ (400 mg) MDA with Magnesium Glasses	ABZ (400 mg) MDA with DOH/DegEd's standard health education	Kato-Katz thick smear	Control arm: baseline prevalence: 20.1%; 1st FU, 16.4%; 2nd FU, 18.8% Intervention arm: baseline prevalence: 25.8%; 1 st FU, 19.7%; 2 nd FU, 21.3%
Hirfanans et al. (2022) ³⁰	Lao PDR	Nam Bak district in Luang Prabang Province	Community-wide, aged 6-60 y	Hookworms, <i>Ascariis</i> , <i>Trichouris</i>	ABZ (400 mg) and IVM (200 µg/kg)	ABZ (400 mg)	qPCR	Control arm: <i>Trichouris</i> ERR, 69%; <i>Trichouris</i> CR, 8%; <i>Ascariis</i> ERR, 100%; <i>Ascariis</i> CR, 100%; hookworms ERR, 99%; hookworms CR, 56% Intervention arm: <i>Trichouris</i> ERR, 99%; <i>Trichouris</i> CR, 66%; <i>Ascariis</i> ERR, 100%; <i>Ascariis</i> CR, 100%; hookworms ERR, 99%; hookworms CR, 59%

Study	Country	Location	Population	STH studied	MDA intervention (dose)	Comparison group	Test used	Key outcomes
Dyer et al. (2023) ³¹	Vietnam	Dak Lak Province	Community-wide and school children	<i>N. americanus</i>	ABZ (400 mg) to school children and the community-wide	ABZ (400 mg) to school children only	Multiplex qPCR	Control arm: Baseline prevalence: 16.7%; EPG, 1678.4 12-mo FU: prevalence: 11.7%; EPG, 1622.0 Relative reduction: prevalence: 30.1%; EPG, 3.4% Intervention arm: Baseline prevalence: 11.7%; EPG, 1699.4 12-mo FU: prevalence: 7.7%; EPG, 747.7 Relative reduction: prevalence: 34.6%; EPG, 56.0%

Study	Country	Location	Population	STH studied	MDA intervention (dose)	Comparison group	Test used	Key outcomes
Weische et al. (2024) ²²	Laos	Nambok district, Luang Prabang	Community-wide	Trichouris	ABZ (400 mg) and IVM (200 µg/kg)	ABZ (400 mg)	Kato-Katz thick smear	Control arm: arithmetic ERR, 0–64.1%; geometric ERR, 56.2%–80.2% Intervention arm: arithmetic ERR, 91.5%–97.2%; geometric ERR, 98.9%–99.1%

ABZ, albendazole; CR, cure rate; DEC, diethylcarbamazine; EPG, egg per gram; ERR, egg reduction rate; FECR, fecal egg count reduction; FU, follow-up; IVM, ivermectin; MDA, mass drug administration; MBZ, mebendazole; PYR, pyrantel pamoate; STH, soil-transmitted helminth
WASH, water, sanitation and hygiene.

Table 2. Results from quasi-experimental studies included in this review

Study	Country	Location	Population	STH studied	MDA intervention (dose)	Comparison group	Test used	Key outcomes
Moose et al. (2013) ²³	Philippines	Municipalities of Mambajao, Catarman, Guinsiliban, Mahinog and Sabay in Camiguin Province, and Gingoog City in Misamis Oriental Province	First-grade students (aged 6–7 y) of public elementary schools	Total STHs	ABZ (400 mg), daily, supervised brushing with a fluoride toothpaste	External concurrent control groups and internal non-concurrent control groups	Kato-Katz thick smear	Intervention arm: 10.7% reduction in the prevalence of moderate-to-heavy STH infections Control arm: 12.4% reduction in the prevalence of moderate-to-heavy STH infections
Park et al. (2016) ²⁴	Indonesia	Gasuggan sub-district, Semarang, Central Java	Children aged 3–11 y	Total STHs	ABZ (400 mg), BALatras	No intervention	Sodium nitrate focal flotation technique	STH prevalence: control arm, 20.4%; intervention arm, 4%

Study	Country	Location	Population	STH studied	MDA intervention (dose)	Comparison group	Test used	Key outcomes
Nachawong et al. (2024) ²⁵	Thailand	Opikai District, Chiang Mai Province	Community-wide	<i>Ascaris</i> and <i>Trichuris</i>	ABZ (400 mg) with community health education	ABZ (400 mg)	Formalin ethyl acetate concentration method	Control arm: Baseline prevalence: <i>Ascaris</i> , 0.6%; <i>Trichuris</i> , 1.8% 3-mo FU prevalence: <i>Ascaris</i> , 0%; <i>Trichuris</i> , 1.3% 6 mos FU prevalence: <i>Ascaris</i> , 0%; <i>Trichuris</i> , 0.5% Intervention arm: Baseline prevalence: <i>Ascaris</i> , 0.8%; <i>Trichuris</i> , 5.4% 3 mos FU prevalence: <i>Ascaris</i> , 0%; <i>Trichuris</i> , 4.8% 6 mos FU prevalence: <i>Ascaris</i> , 0.9%; <i>Trichuris</i> , 6.4%

ABZ, albendazole; CR, cure rate; DEC, diethylcarbamazine; EPG, egg per gram; ERR, egg reduction rate; FU, follow-up; IVM, ivermectin; MDA, mass drug administration; MBZ, mebendazole; PYR, pyrantel pamoate; STH, soil-transmitted helminth; WASH, water, sanitation and hygiene

Table 3. Results from cross-sectional studies included in this review

Study	Country	Location	Population	STH studied	MDA history	Test used	Key outcomes
Coelan et al. (2012) ²⁶	Lao PDR	Oudomxay, Luangphabang, Houphane and Xiangkhouang Provinces	Community-wide, 6–11-y-old children, women of childbearing age	<i>Ascaris</i> , <i>Trichuris</i> , Hookworms, <i>Strongyloides</i>	ABZ (400 mg)	Formalin-ether concentration	STH prevalence: total STH, 70.6%; <i>Ascaris</i> , 26.1%; <i>Trichuris</i> , 41.5%; hookworms, 46.3%; <i>Strongyloides</i> , 8.9%
Sanza et al. (2013) ²⁷	Philippines	Aklan, Antique and Capiz	Third-grade students aged 8–9 y	<i>Ascaris</i> , <i>Trichuris</i>	MBZ (500 mg)	Kato-Katz thick smear	Prevalence of moderate-to-heavy STH infections significantly decrease from the baseline Effect on egg counts: decreased within the first follow-up but increased in the last follow-up
Belizario et al. (2016) ²⁴	Philippines	Municipalities of Arroyo, Cawayan, Milagros and Montreal in Masbate Province	Preschool-age children	Hookworms, <i>Ascaris</i> , <i>Trichuris</i>	10 years of MDA with ABZ (400 mg) or MBZ (500 mg)	Kato-Katz thick smear	STH prevalence: <i>Ascaris</i> , 59.0%; <i>Trichuris</i> , 54.0%; hookworms, 2%

Study	Country	Location	Population	STH studied	MDA history	Test used	Key outcomes
Dunn et al. (2017) ²³	Myanmar	Udo village, Taikkyi township, Yagon Region and Kyee Kan Theik village, Nyaung Don township, Ayeeyarwady Region	Community-wide	Hookworms, <i>Ascaris</i> , <i>Trichuris</i>	ABZ (400 mg), DEC (6 mg/kg)	Kato-Katz thick smear	STH prevalence: total STHs, 27.81%; <i>Ascaris</i> , 5.34%; <i>Trichuris</i> , 18.12%; hookworms, 8.71%
Ash et al. (2017) ²⁶	Lao PDR	Phongsaly Province	Community-wide	Hookworms, <i>Ascaris</i> , <i>Trichuris</i>	2 rounds of MDA with triple ABZ (400 mg) 5 mos apart	McMonz-ore counting method, PCR species identification	MDA1 STH prevalence reduction: <i>Ascaris</i> , 95.6%; <i>Trichuris</i> , 69.2%; hookworms, 83.4% STH ERR: <i>Ascaris</i> , 99.4%; <i>Trichuris</i> , 77.2%; hookworms, 94.4% MDA2 STH prevalence reduction: <i>Ascaris</i> , 93.5%; <i>Trichuris</i> , 61%; hookworms, 84.5% STH ERR: <i>Ascaris</i> , 99.3%; <i>Trichuris</i> , 88.5%; hookworms, 97.8%

Study	Country	Location	Population	STH studied	MDA history	Test used	Key outcomes
Stool et al. (2018) ¹¹	Indonesia	Nangapanda Village, Ende District, Flores Island	School children	Hookworms, <i>Ascaris</i> , <i>Trichuris</i>	ABZ (400 mg)	Kato-Katz thick smear	Baseline STH prevalence: <i>Ascaris</i> , 8.4%; <i>Trichuris</i> , 11.2%; hookworms, 4.9% FU STH prevalence: <i>Ascaris</i> , 1.0%; <i>Trichuris</i> , 3.9%; hookworms, 1.0%
Vaz Nery et al. (2018) ²²	Timor Leste	Manufahi municipality	Community-wide	<i>Necator</i> , <i>Ascaris</i>	ABZ (400 mg)	Real-time multiplex PCR	<i>Necator americanus</i> CR, 58.3%; infection intensity reduction: 88.9% <i>Ascaris</i> CR, 91.4%; infection intensity reduction, 95.5%
Sungkar et al. (2018) ²⁵	Indonesia	Persahabatan Village, Southwest Sumba District	Children aged 1–15 y	Hookworms, <i>Ascaris</i> , <i>Trichuris</i>	Triple dose ABZ (400 mg)	Kato-Katz thick smear	Baseline STH prevalence: <i>Ascaris</i> , 65.4%; <i>Trichuris</i> , 55.8%; hookworms, 15.4% FU STH prevalence: <i>Ascaris</i> , 8.3%; <i>Trichuris</i> , 12.8%; hookworms, 4.0%

Study	Country	Location	Population	STH studied	MDA history	Test used	Key outcomes
Dunn et al. (2015) ⁴⁶	Myanmar	Udo village, Taikkyi township, Yangon Region and Kyeo Kan Thaik village, Nyaung Don township, Ayeyarwady Region	Community-wide	Hookworms, <i>Ascaris</i> , <i>Trichuris</i>	Trice administration of ABZ (400 mg) 4 and 6 mos apart	Kato-Katz thick smear	Baseline STH prevalence: <i>Ascaris</i> , 5.5%; <i>Trichuris</i> , 17.02%; hookworms, 9.75% 1st FU STH prevalence: <i>Ascaris</i> , 1.72%; <i>Trichuris</i> , 11.82%; hookworms, 6.69% 2nd FU STH prevalence: <i>Ascaris</i> , 2.49%; <i>Trichuris</i> , 11.85%; hookworms, 5.54%
Chai et al. (2020) ⁴⁷	Myanmar	South Dagon, North Dagon, and Hlaing-tharyar of Yangon Region	School children	Hookworms, <i>Ascaris</i> , <i>Trichuris</i>	ABZ (400 mg)	Kato-Katz thick smear	STH prevalence: <i>Ascaris</i> , 17.2%; <i>Trichuris</i> , 19.4%; hookworms, 0.2%
Stracke et al. (2021) ⁴⁸	Thailand	Mae Song subdistrict, Tha Song Yang district, Tak Province	Pre-school and school-aged children aged 2-6 y	<i>Ancylostoma certanthemum</i> , <i>Ascaris</i> , <i>Trichuris</i>	Annual MBZ (500 mg) administration	multiplexed-tandem qPCR	STH prevalence: <i>Ascaris</i> , 39.2%; <i>Trichuris</i> , 36.6%; <i>A. ceylanicum</i> , 1.1%

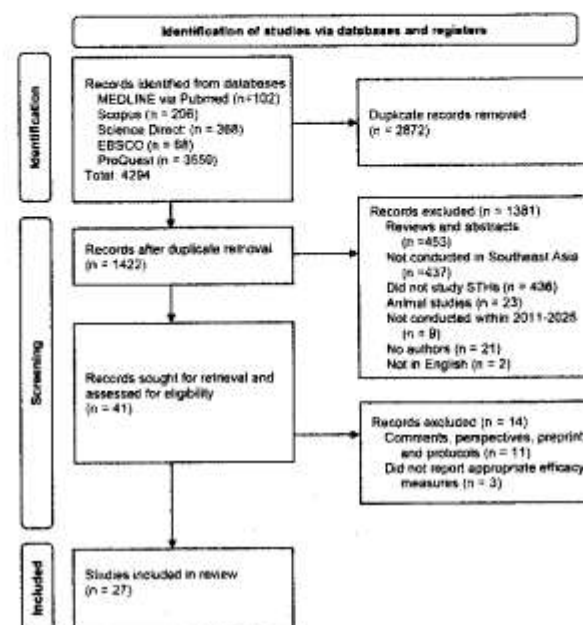
Study	Country	Location	Population	STH studied	MDA history	Test used	Key outcomes
Colella et al. (2021) ⁴⁷	Cambodia	Chambak Pa'ven, Chamlong, Kam-pot, Rovieng, Thong-Sanket, Rong, Pal Hin, Anglong Svay, Chamlong, Bos Pey and Sm'er Thom, Rovieng district, Preah Vihear Province	Community-wide and animals	<i>A. ceylanicum</i> , <i>Necator americanus</i>	ABZ (400 mg)	multiplex qPCR	All hookworms: CR, 46.4%; geometric ERR, 83.2%; arithmetic ERR, 72.5% <i>A. americanus</i> : CR, 48.3%; geometric ERR, 94.5%; arithmetic ERR, 77.6% <i>A. ceylanicum</i> : CR, 52.6%; geometric ERR, 98.7%; arithmetic ERR, 62.9%

Study	Country	Location	Population	STH studied	MDA history	Test used	Key outcomes
Hoog et al. (2021) ⁴⁸	Myanmar	Shwe Pyi Thar, Twantay, and Kyauktan, Yangon Region	Primary school-children	Hookworms, <i>Ascaris</i> , <i>Trichuris</i>	9 rounds of ABZ (400 mg)	Kato-Katz thick smear	1st FU prevalence: <i>Ascaris</i> , 23.3%; <i>Trichuris</i> , 23.3%; hookworms: 0.15% 2nd FU prevalence: <i>Ascaris</i> , 17.3%; <i>Trichuris</i> , 26.2%; hookworms: 0.8% 3rd FU prevalence: <i>Ascaris</i> , 17.3%; <i>Trichuris</i> , 27.4%; Hookworms, 0.3% 4th FU prevalence: <i>Ascaris</i> , 2.9%; <i>Trichuris</i> , 21.3%; hookworms, 0% 5th FU prevalence: <i>Ascaris</i> , 7.2%; <i>Trichuris</i> , 14.5%; hookworms, 0.2% 6th FU prevalence: <i>Ascaris</i> , 3.6%; <i>Trichuris</i> , 20.2%; hookworms, 0.1%

Study	Country	Location	Population	STH studied	MDA history	Test used	Key outcomes
Tee et al. (2022) ⁴⁹	Malaysia	Rasau (Perak state), Sungai Juloh, Tanjung Sepat and Bukit Bangkon, Selangor States	Community-wide, aged 4-85 y	Hookworms, <i>Ascaris</i> , <i>Trichuris</i>	3-d course of ABZ (400mg)	Modified Kato-Katz technique	3-wk FU: <i>Ascaris</i> : CR, 100%; ERR, 100% <i>Trichuris</i> : CR, 64.6%; ERR, 84.2% Hookworms: CR, 100%; ERR, 100% 6-wk FU: <i>Ascaris</i> : no infection <i>Trichuris</i> : 25.6% prevalence; 250.2 EPG Hookworms: no infection
Le et al. (2023) ⁵⁰	Timor Leste	Dili, Ermera, and Manufahi municipalities	Schoolchildren	<i>Ascaris</i> , <i>Trichuris</i> , <i>N. americanus</i> , <i>A. ceylanicum</i> , <i>Acyllostoma dandenale</i>	IVM (200 µg/kg), DEC (6 mg/kg), ABZ (400 mg)	Multiplex qPCR	Baseline prevalence: <i>Ascaris</i> , 18.1%; <i>Trichuris</i> , 4.8%; <i>N. americanus</i> - 7.4%; <i>A. ceylanicum</i> - 0.6%; <i>A. dandenale</i> , 0.2% 18 mos FU prevalence: <i>Ascaris</i> , 13.2%; <i>Trichuris</i> , 0.6%; <i>N. americanus</i> - 5.9%; <i>A. ceylanicum</i> - 3.4%; <i>A. dandenale</i> , 0%

ABZ, albendazole; CR, cure rate; DFC, diethylcarbamazine; EPG, egg per gram, ERR, egg reduction rate; FU, follow-up; IVM, ivermectin; MBZ, mebendazole; MDA, mass drug administration; STH, soil-transmitted helminth.

Figure 1





Unraveling the mechanisms of benzimidazole resistance in hookworms: A molecular docking and dynamics study

Jan Clyden B. Tenorio^{a,1}, Muhammad Fikri Helkal^{b,1}, Alok Kafle^c,
Mark Andrian B. Macalalad^{d,1}, Predmoore L. Oroso^{e,1}, Prasert Saichua^{f,1},
Sutas Suttiprapa^{g,1}

^a Tropical Medicine Graduate Program, Department of Tropical Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand

^b Department of Veterinary Parasitology Systems, College of Veterinary Medicine, University of Southern Mindanao, Kidogo, 9407 Compostela, Philippines

^c Virology and Vaccine Research and Development Program, Department of Science and Technology - Industrial Technology Development Institute, 1631 Tagaytay City, Marikina, Philippines

^d iGAT Fellow Program, Department of Science and Technology, 1631 Tagaytay City, Marikina, Philippines

^e Department of Biology, College of Arts and Sciences, University of the Philippines - Manila 1000 Manila, Marikina, Philippines

^f Department of Tropical Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand

^g WHO Collaborating Center for Research and Control of Opisthorchiasis (Southeast Asian Liver Fluke Disease), Tropical Disease Research Center, Khon Kaen University, Khon Kaen 40002, Thailand

ARTICLE INFO

Keywords:
Anthelmintic Resistance
Deworming
Ankylostomes
Necator
Registered Tropical Diseases

ABSTRACT

Background: Benzimidazole resistance is an emerging challenge among parasitic helminths. It is caused by single nucleotide polymorphisms (SNPs) in specific loci in helminth β -tubulin genes. Field studies and laboratory investigations reported resistance-associated SNPs in 4 codon locations with 7 allelic variations among hookworms. This study aimed to determine the effects of these mutations on the binding efficiency and behavior of the β -tubulin protein in four hookworm species against four benzimidazole drugs.

Methods: β -tubulin gene coding sequences of *Ancylostoma ceylanicum*, *A. thaidense*, *A. ceylanicum*, and *Necator americanus* were retrieved, annotated phylogenetically, and used to construct the 3D structure models of the proteins. The modeled protein structures were verified and edited to contain the reported SNPs: Q134H, F167Y, E198A, E198K, E198V, F200Y, and F200Y. Benzimidazole drugs such as albendazole (ABZ), fenbendazole (FBZ), mebendazole (MBZ), and oxfendazole (OFZ) were used as ligands. Molecular docking experiments were performed with the wild-type and mutated proteins. Molecular dynamics simulation assessed the dynamic behavior of the β -tubulin-benzimidazole complex.

Results: *In silico* docking assessments showed that various amino acid substitutions due to resistance-associated SNPs cause alterations in binding affinities and positions. E198K and Q134H in hookworm β -tubulin substantially weakened the binding affinities and altered the binding positions of benzimidazole drugs. Molecular dynamics analysis revealed that these mutations also caused marked reductions in the binding free energies owing to diminished hydrogen bond reactivity with the benzimidazole ligands.

Conclusion: The evidence shown herein indicates that mutation at positions 134 and 198 are detrimental to conferring benzimidazole resistance among hookworms. The presence of these mutations may alter the efficacy of pharmacological interventions. Hence, further studies should be conducted to assess their emergence among hookworms in endemic areas with histories of chemotherapy.

* Corresponding author at: Department of Tropical Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand.

E-mail address: jan@pitt.tmu.ac.th (J.C.B. Tenorio).

¹ <https://orcid.org/0000-0002-2511-2952>

² <https://orcid.org/0000-0002-4672-8107>

³ <https://orcid.org/0000-0001-9613-0163>

⁴ <https://orcid.org/0000-0002-8861-7923>

⁵ <https://orcid.org/0000-0002-4548-0374>

<https://doi.org/10.1016/j.jgeb.2024.100473>

Received 26 August 2024; Received in revised form 26 December 2024; Accepted 11 February 2025

Available online 17 February 2025

1687-137X/© 2025 The Author(s). Published by Elsevier Inc. on behalf of Academy of Scientific Research and Technology. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Hookworm infections continue to pose a significant threat to public and veterinary health worldwide. Human hookworm infections are primarily caused by *Necator americanus* and *Ancylostoma duodenale*, while *A. ceylanicum*, *A. braziliense*, and *A. tubaeforme* are the primary zoonotic agents of hookworm infections in animals. *A. ceylanicum* is a true zoonotic hookworm widespread in countries within the Asia-Pacific region. Hookworm infections impose a substantial burden on endemic populations, with an estimated loss of 800,000–4 million disability-adjusted life years. Moreover, zoonotic hookworm infections cause approximately 239–590 million cases globally. Among animals, hookworms reportedly infect approximately 41 % of domestic dogs in the Asian continent, with *A. ceylanicum* and *A. tubaeforme* accounting for 27 % and 24 % of these infections, respectively. To abate the health threats caused by hookworm infections, numerous control efforts have been mobilized by the public health and veterinary sectors in many countries. Preventive chemotherapy for humans and routine deworming for animals continue to be the cornerstones of hookworm control programs. The repeated and sustained administration of benzimidazole drugs, such as albendazole, mebendazole, and fenbendazole, has raised concerns about the development of benzimidazole resistance in soil-transmitted helminths, including hookworms. Drug-resistant helminths pose significant challenges to public and veterinary health. These resistant parasites can cause more severe infections and spread more easily. Additionally, the limited availability of effective drugs to treat these infections further exacerbates the problem.

Single nucleotide polymorphisms (SNPs) in the β -tubulin isotype 1 gene result in benzimidazole resistance in helminths. Canonical nucleotide mutations F157Y (TTC,TTT to TACT,AT), E198A (GAG,GAA to GCG,GCA), and F200Y (TTC,TTT to TACT,AT) cause amino acid substitutions that change the structure of the β -tubulin protein, thereby adversely affecting its binding efficiency with benzimidazole drug ligands. These mutations have been reported in different hookworm species that affect both humans and animals. Recently, multidrug-resistant *A. ceylanicum* isolates that carry such mutations have emerged in the United States and Canada. Moreover, studies on these multidrug-resistant hookworms have resulted in the discovery of novel SNPs that have not been previously found among members of *Ancylostoma*: Q134H (CAA to CAT), E198K (GAG, GAA to AAG, AAA) and E198V (GAG, GAA to GTA). Laboratory investigations that induced resistance in *A. ceylanicum* have produced inconsistent findings regarding the correlation between these SNPs and benzimidazole resistance. One of these laboratory studies identified F200L (TTC,TTT to TTA) mutation which was linked to the induced resistance. Despite these reports, the exact mechanisms underlying benzimidazole resistance in hookworms remain unclear and require further study.

Recent *in silico* studies determined the key interactions between helminth β -tubulin and benzimidazole drugs. However, these studies focused on the use of albendazole as the sole drug ligand. This seems limiting since albendazole is not frequently used against canine or feline hookworms, where fenbendazole and oxfendazole are used in deworming preparations. Moreover, these studies have not explored the effects of other SNPs reported recently in addition to the canonical mutations. Therefore, to gain novel insights into benzimidazole resistance, this study aimed to determine the effects of all SNPs reported in hookworms on the binding behavior and efficacy of hookworm β -tubulin isotype 1 protein with several benzimidazole drugs. Here, we report the effects of resistance-associated mutations in the hookworm β -tubulin proteins when docked *in silico* with benzimidazole ligands.

2. Materials and methods

2.1. Hookworm β -tubulin nucleotide and amino acid sequences

As described previously by Jones et al., four isotypes of β -tubulin exist among the members of *Ancylostomidae* (Supplementary Table 1). Among hookworms of public and veterinary health concerns, the coding nucleotide and amino acid sequences of these proteins have only been available for *A. ceylanicum*, *A. duodenale*, and *N. americanus*. These β -tubulin sequences, both nucleotide and amino acids, were obtained from WormBase Parasite. Moreover, the amino acid sequences were confirmed by searching UniProt.

2.2. Phylogenetic analysis

BiEdit Sequence Alignment Editor version 7.2.5 was used to align both nucleotide and amino acid sequences. The sequences were aligned using ClustalW Multiple Alignment function in BiEdit. The aligned sequences were trimmed using the alignment editor of MEGA version 11. The best model was determined for both the trimmed nucleotide and amino acid sequences, and phylogenetic trees were constructed. Maximum likelihood trees were drawn based on the K2 (Kimura-2 parameter) + G model for the nucleotide sequences and JTT (Jones-Taylor-Thuntan) model for amino acid sequences with 1000 bootstraps in MEGA version 11.

2.3. Homology Modeling and quality assurance checks

β -tubulin proteins of the four hookworm species were modeled using SWISS-MODEL (<https://www.swissmodel.org>) [Accessed: 27 May 2024]. The amino acid sequences were used for protein structure modeling, with a previously described β -tubulin (PDB ID No. 6B3) as the template. 6B3 was selected because it contains β -tubulin proteins (chains B and D), whose crystal structures were determined with a ligand bound to the colchicine binding site in a similar manner to benzimidazole drugs. The percentage of sequence identity similarity and GMQS (Global Model Quality Estimate) scores were noted (Supplementary Table 2). Modeled β -tubulins were submitted to various platforms for quality assurance checks. Before downloading the PDB file of the homology-modeled protein, the Ramachandran plot, which gives the percentage of amino acids in their favored conformation in SWISS-MODEL's structure analysis function, was inspected and noted. Moreover, the modeled proteins were submitted to the UCLA-DOE LAB — SAVES v6.0 (<https://saves.uclaf.edu>) [Accessed: 27 May 2024], and the results of the PROCHECK Ramachandran plots and VERIFY3D plots were recorded (Supplementary Table 3).

Since mutations occurring in the β -tubulin isotype 1 gene are the cause of benzimidazole resistance, modeled isotype 1 proteins were selected for *in silico* docking assessments with benzimidazole drugs. Known resistance-associated SNPs at positions 134, 167, 198, and 200: Q134H, F167Y, E198A, E198K, E198V, F200Y, and F200L were introduced into amino acid sequences and were used to model proteins using SWISS-MODEL. These mutations were identified through a prior systematic review that our team conducted. All mutations that we reported in the systematic review were included in this study. These mutated proteins were also assessed using PROCHECK and VERIFY3D (Supplementary Table 4). Both wild-type and mutated hookworm β -tubulin were evaluated for their structural similarity to the mammalian template using the PDB Pairwise Structure Alignment (<https://www.rcsb.org/alignment>) [Accessed: 29 May 2024]. The mammalian β -tubulin monomer (PDB ID No. 1F7J) was used as the template. RMSD, TM Score, Identity %, Aligned residues, and sequence lengths were documented and assessed.

2.4. In silico docking

To obtain the best binding poses and their respective affinity values, in silico docking analyses with the modeled β -tubulin isotype 1 proteins and benzimidazole drugs were performed using Autodock Vina[®] in the PyRx-Virtual Screening Tool version 0.5 (https://github.com/robertodominguez). Before uploading the proteins to PyRx, polar hydrogens were added to all wild-type and mutated β -tubulins using the BIOVIA Discovery Studio Visualizer. These proteins were then used as docking macromolecules for PyRx. 3D ligand structures of Albendazole (as Albendazole sulfoxide, ABZ), Mebendazole (MBZ), Fenbendazole (FBZ), and Oxfendazole (OBZ) were obtained from PubChem[®] (https://pubchem.ncbi.nlm.nih.gov/) (Accessed 25 May 2024). ABZ, MBZ, FBZ, and OBZ were used because these drugs are the most commonly used anthelmintics in clinical treatment and control programs.¹⁶ Also, previous studies of similar methodologies and aims have utilized these drugs.²⁹ These structures were imported to PyRx, set as ligands, and energy-minimized.

In silico docking was performed by setting the center of the search grid cube at position 200, with the surface of the cube at 25 Å from the center (coordinates: X = 15.6; Y = 57.5; Z = 33.1). This search grid configuration ensured that all positions where the SNPs occurred were inside the cube. The binding positions with the highest binding affinities (kcal/mol) and the lowest RMSD scores (lower and upper bounds, preferably 0) were considered the best and were saved as PDB files. The binding poses of the in silico docking experiments were visualized using the Receptor-Ligand Interaction function in BIOVIA Discovery Studio Visualizer version 21.1.0.20298 (https://discover.slb.com/products/3d-visualizer-quickstart-tool). Both the 3D and 2D representations of the docking results were evaluated. The list of interacting amino acids and their frequencies were summarized and recorded (Supplementary Tables 4 and 5).

2.5. Molecular dynamics simulation and trajectory analysis

Docked complexes of the top two resistance-associated mutations that caused the highest reduction in binding affinity were analyzed using molecular dynamics (MD) to determine the binding behavior and binding free energies. MD simulations were performed using GROMACS 2023.2. The topology of the benzimidazole ligands (Albendazole and Fenbendazole) was assigned using the Chemistry at Harvard Macromolecular Mechanics (CHARMM) General Force Field (CGenFF) server (https://www.cgmtools.org/). The protein topology of the Ancylostoma ceylanicum β -tubulin receptor was generated using the CHARMM36 force field (July 2022 version), which was incorporated into GROMACS. The protein complex was simulated inside a cubic box with a 10 Å buffer from the box edge to avoid interactions with the periodic image from the adjacent unit cell. The box was filled with TIP3P water molecules and neutralized with Na and Cl ions. Then, 0.15 M NaCl was added. The system was energy minimized using steepest descent for 50,000 steps to remove steric clashes. It was then equilibrated to 300 K and 1 bar using NVT and NPT ensembles, respectively. Both equilibration methods used 50,000 steps or 100 ps of simulation time. Temperature was regulated using the V-rescale thermostat, while pressure was controlled using the Berendsen barostat. The MD run was conducted for 10,000,000 steps or 100 ns, saving snapshots every 5,000 steps, resulting in a trajectory file composed of 1,000 frames. Hydrogen bonds (H-bonds) were identified based on two criteria: a donor-acceptor distance of no more than 3.5 Å and a donor-hydrogen-acceptor angle of at least 120 degrees. RMSD (Root Mean Square Deviation), RMSF (Root Mean Square Fluctuation), SASA (Solvent Accessible Surface Area) plot, and H-bond plots were constructed to assess the behavior of the β -tubulin-benzimidazole ligand complexes. The equilibration point was determined to be beyond the 80 ns mark of the simulation.

The absolute binding free energies, determined through MM-GBSA (Molecular Mechanics-Generalized Born Surface Area) and MM-PBSA (Molecular Mechanics-Poisson-Boltzmann Surface Area) methods,

were calculated using the last 20 ns of the simulations. MM-GBSA and MM-PBSA estimates were derived using Equation 1. ΔG_{GBSA} represents the sum of electrostatic and van der Waals energies, ΔG_{Solv} is the sum of polar and nonpolar solvation energy, and $-\Delta T\Delta S$ represents the interaction entropy calculated from the last 25 frames (Supplementary Tables 6 and 7). The MM-GBSA and MM-PBSA methods employed the Lennard-Jones and Coulombic potentials for van der Waals and electrostatic energy calculations, respectively. MM-GBSA employed the Generalized Born model to estimate the polar solvation energy, while MM-PBSA used the Poisson-Boltzmann model. Nonpolar solvation energies were assumed to have a linear dependency on the SASA values. Per-residue binding free energy decomposition was done using the MM-GBSA method. Binding free energies were calculated using the last 20 ns of the simulation after the equilibration point was reached.

$$\Delta G_{bind} = \Delta G_{GBSA} + \Delta G_{Solv} - T\Delta S \quad (1)$$

3. Results

3.1. Hookworm β -tubulin and their phylogeny

All modeled β -tubulin passed the quality assurance checks based on the constructed Ramachandran Plots in SWISS-MODEL and PROCHECK (Supplementary Table 1). However, the modeled isotype 3 β -tubulin of *A. caninum*, isotype 4 of *N. americanus*, and both isotypes 3 and 4 of *A. suadense* failed the VERIFY3D check (i.e., the modeled protein scored less than 80%). All isotype 1 β -tubulin of all hookworm species mutated to contain the reported SNPs passed quality assurance checks (Supplementary Table 2). These results justify the use of only isotype 1 β -tubulins in the in silico docking experiments. In addition, the alignment of both coding nucleotide and amino acid sequences revealed that in all species and isotypes, the positions holding the SNPs related to benzimidazole resistance had very similar codon constitutions, resulting in similar sets of amino acids (Fig. 1).

The cladograms derived from the phylogenetic analysis of nucleotide coding (Fig. 1A) and amino acid sequences (Fig. 1B) revealed that hookworm β -tubulins clustered based on their respective isotypes. Isotypes 1 and 2 in all species were more evolutionarily related, and the same was true for isotypes 3 and 4. As expected, the β -tubulins of *N. americanus* were genetically distant from those of the three *Ancylostoma* species based on the cladograms from the nucleotide sequences. Conversely, based on the tree derived from the amino acid sequences, *A. suadense* β -tubulin isotype 4 was genetically distant from those of *A. caninum*, *A. ceylanicum*, and *N. americanus*. Moreover, based on the same tree, isotypes 3 and 4 showed greater evolutionary divergence than isotypes 1 and 2. The pairwise structural alignment of the predicted hookworm β -tubulin proteins revealed that all structure predictions had high structural similarity to the defined β -tubulin crystal structure from PDB ID No. 1JH (Supplementary Fig. 1).

3.2. R2 resistance mutations affect benzimidazole binding affinities

The in silico docking analysis revealed varying binding affinities between different hookworm species and benzimidazole drugs (Fig. 2). Among the β -tubulin isotype 1 protein across all species, MBZ had the highest binding affinity, while ABZ had the lowest. Moreover, the binding affinities of the different benzimidazole ligands were lowest in *N. americanus*; the decrease was particularly marked for FBZ, OBZ, and ABZ reflecting the results of the phylogenetic analysis. Mutations of the β -tubulin through the introduction of the reported SNPs resulted in marked alterations in binding affinities. Overall, the Q134H and E198K mutations markedly decreased the binding affinities of the hookworm β -tubulins, regardless of the interacting benzimidazole drug. In addition, a similar but less reduction in binding affinity was observed when β -tubulins had the F200L mutation; the opposite was observed in proteins with the F200Y mutation. In contrast, the other mutations caused

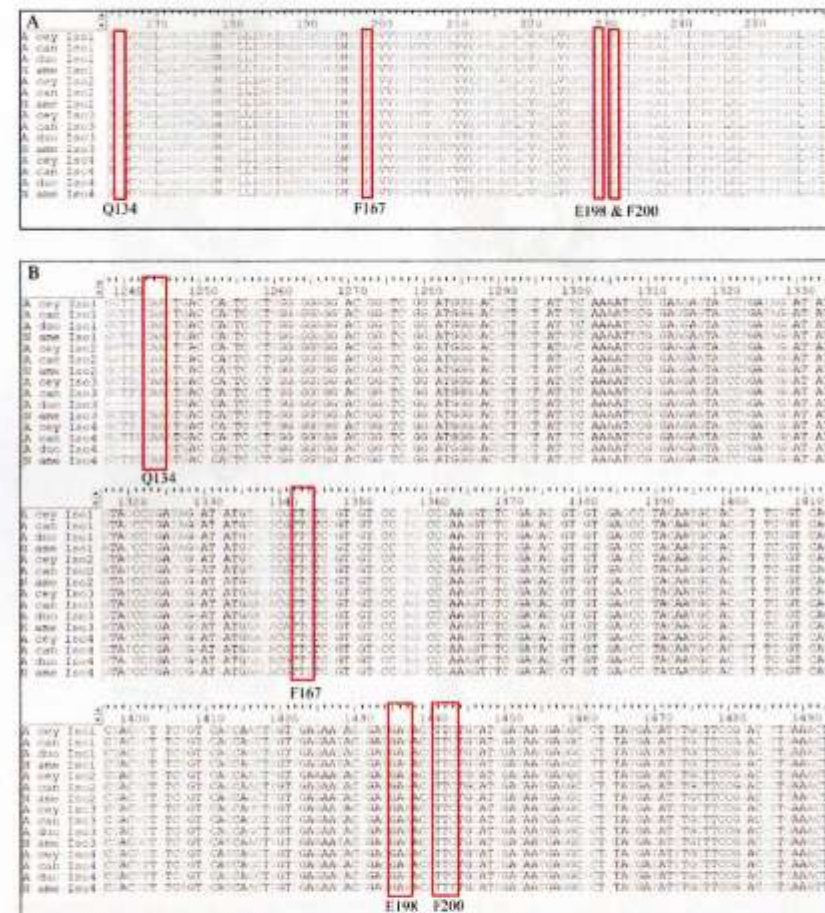


Fig. 1. Sequence alignment of the hookworm β -tubulin amino acids (A) and nucleotide sequences (B). The amino acid and codon positions carrying SNPs related to benzimidazole resistance are highlighted and labeled. Alignments revealed similar sequence profiles across different β -tubulin isotypes and hookworm species. Sequence alignment was performed using ClustalW Multiple Alignment in the BioEdit Sequence Alignment Editor version 7.2.2.5.

either slight increases or unchanged docking binding affinities.

3.3. R2 resistance mutations impact the binding positions and interacting residues

In addition to the altered binding affinities, SNPs also affected the

binding pose between the β -tubulin proteins and benzimidazole drugs (Fig. 3). The observed reduction in binding affinities of hookworm β -tubulins with E198K and Q134H was supported by alterations in the docking poses of the protein and benzimidazole ligands. The loss of key carbon-hydrogen bonds was observed when glutamine was swapped with histidine at position 134. However, this mutation resulted in the

3.4. Molecular dynamics simulation

To further investigate the results of the molecular docking experiments, hookworm β -tubulin with the E198K and Q134H mutations complexed with FBZ and ABZ were analyzed using molecular dynamics simulations. Overall, the hookworm β -tubulin, both wild-type and mutated, and benzimidazole ligand complexes stabilized around 30 ns and continued beyond the equilibration point at 80 ns (Fig. 5A and B). The wild-type, E198K, and Q134H-ABZ complexes have comparable RMSD values post-equilibration point (wildtype = 2.42 ± 0.06 Å, E198K = 2.26 ± 0.09 Å, Q134H = 2.51 ± 0.08 Å). A similar trend was observed when the β -tubulins were docked with FBZ (wildtype = 2.21 ± 0.09 Å, E198K = 2.52 ± 0.07 Å, Q134H = 2.26 ± 0.08 Å) over the 100 ns simulation. The RMSF values showed that the complexes shared a similar profile of flexible regions, regardless of the mutations. Among the β -tubulins complexed with ABZ, flexible regions included residues

37–45, 94–99, and 274–284 (Fig. 5C). For those docked with FBZ, increased flexibility was observed at residues 173–180, 244–253, and 275–282 (Fig. 5D). The Solvent-Accessible Surface Area (SASA) plots (Fig. 5E and F) revealed that both the wild-type and mutant proteins maintained similar levels of solvent exposure when bound to ABZ (wildtype = 1.87 ± 0.02 Å², E198K = 1.79 ± 0.02 Å², Q134H = 1.84 ± 0.02 Å²) and FBZ (wildtype = 1.81 ± 0.03 Å², E198K = 1.81 ± 0.02 Å², Q134H = 1.82 ± 0.04 Å²) after the equilibration point was reached. This suggests that the overall structural conformations of the protein were not significantly altered by the mutations or the binding of the BZ ligand. The mutations and ligand binding did not induce substantial changes in the protein's overall shape or flexibility. The RMSF, RMSD, and SASA plots reveal that the wild-type and mutated β -tubulin-benzimidazole complexes share similar dynamic behaviors.

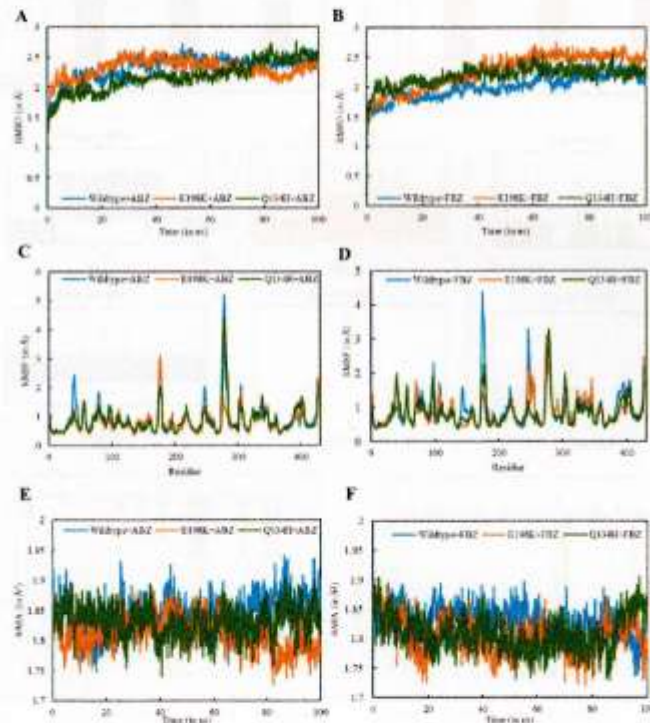


Fig. 5. RMSD (Root Mean Square Deviation) and RMSF (Root Mean Square Fluctuation) of the β -tubulin-benzimidazole complexes. The β -tubulin ABZ (A) and β -tubulin-FBZ (B) complexes show similar dynamic behavior. Moreover, complexes with Albendazole (C) and Fenbendazole (D) show dynamic flexible regions in the β -tubulin backbone. The SASA (Solvent-Accessible Surface Area) (E and F) plots showed that both wildtype and mutant protein showed comparable area that were solvent exposed across the simulation time, indicating similar structural conformations regardless of the mutation and BZ ligand used. The RMSF, RMSD, and SASA plots reveal that the wild-type and mutated β -tubulin-benzimidazole complexes share similar dynamic behaviors.

3.5. E198K and Q134H reduce the binding efficiency of β -tubulin-BZ complex

The binding free energy is used to estimate the energetic differences between the bound and unbound states of the protein-ligand complexes, which can be used to quantify the stability and favorability of binding. In this study, the MM-PBSA and MM-GBSA methods were used to estimate the binding free energy between the β -tubulin and benzimidazole ligands (Supplementary Tables 6 and 7). The results showed that complexes with E198K and Q134H mutations in β -tubulins showed reduced binding energies compared to those with the wild-type (Fig. 6A). Regardless of the benzimidazole ligand and binding free energy determination method, the Q134H mutation caused significant decreases in binding energies, thereby causing weaker interactions. Hydrogen bonds between the β -tubulin receptors, both wild-type and mutated, and the benzimidazole drug ligands were determined during molecular dynamics simulations (Fig. 6B). Overall, more H-bond contacts were formed between the wild-type and ABZ and FBZ. A decrease in the number of H-bonds was observed when the β -tubulin containing the E198K mutation interacted with ABZ. This decrease was more pronounced when the β -tubulin had the Q134H mutation. Using FBZ as a ligand, marked reductions in the number of H-bonds were observed when E198K and Q134H mutations were present in the β -tubulin receptor. These results indicate that the E198K and Q134H mutations cause weaker binding interactions with benzimidazole drugs indicating their role in benzimidazole resistance among hookworms. The per-residue energy decomposition analysis (Fig. 6C) indicated that amino acids at positions 134, 167, 198, and 200, which were mutation sites, were involved in the binding interaction. Moreover, the strongest interactions were observed in residues 226–248, 251–258, 315–318, 350, and 368. The latter interacting residues correspond to the positions that were frequently interacting with the BZ ligands in the docking experiments. The results depicted in Fig. 6D showed that the reduction in binding free energies among the complexes with the β -tubulin mutations is caused by a diminished number of hydrogen bonds between the protein and the ligand. Molecular dynamics simulations proved that the E198K and Q134H mutations in hookworm β -tubulins confer benzimidazole resistance. Likewise, other interacting amino acid positions may also harbor mutations that tend to undermine the binding efficiency of the β -tubulin-BZ drug complex.

4. Discussion

Benzimidazole resistance is an emerging threat that can potentially undermine efforts to control and eliminate STH, including hookworms. Despite concerns raised recently, the exact mechanism of resistance among these parasites remains unclear. In this study, we used *in silico* docking with open-source platforms to study the potential consequences of SNPs in the β -tubulin isotype 1 gene of several hookworm species. Phylogenetic analysis revealed that the hookworm β -tubulin nucleotide and amino acid sequences clustered within their specific isotypes. The *in silico* docking analysis revealed that amino acid substitutions brought about by SNPs in 4 codon locations of the hookworm β -tubulin isotype 1 protein resulted in altered binding affinities and binding positions. Among these mutations, E198K and Q134H may be important in conferring benzimidazole resistance. Molecular dynamics analysis revealed that the inclusion of these mutations did not significantly alter the binding dynamics of the β -tubulin-benzimidazole complex. However, the E198K and Q134H mutations caused marked reductions in the H-bonds formed, which resulted in significant reductions in the binding free energy. These results indicated that these mutations confer benzimidazole resistance.

The clustering of various β -tubulin genes based on their isotypes observed in this study was similar to those reported previously. Reconstruction of *Ascaris suum* phylogenies based on β -tubulin genes showed that ascaris β -tubulins were clearly separated into seven known

isotypes (isotypes A–G). This clustering is believed to have arisen early in the evolution of various roundworm species, such as *Ascaris lumbricoides*, *Anisakis simplex*, and *Parascaris equorum*, within the Ascaridomorpha infraorder. Our results, which indicated that hookworm β -tubulin isotypes 1 and 2 are monophyletic but distinct from isotypes 3 and 4, which are also closely related, are also echoed by the results of Jones et al. They observed a similar clustering of strongyle β -tubulins and revealed a similar phylogenetic profile for other important parasitic nematodes, such as *Haemonchus contortus* and *Trichostrongylus axei*. The results of our phylogenetic analysis and the aforementioned results from other published studies may have implications for benzimidazole resistance. Since benzimidazole resistance is often linked to SNPs in the β -tubulin isotype 1 gene of nematodes, its close monophyletic relationship with isotype 2 suggests that SNPs occurring in the latter may also confer or assist in the emergence of benzimidazole resistance. This was previously observed by Doyle et al. when they studied resistant *H. contortus*. They observed that increasing Thiabendazole EC₅₀ concentrations correlated well with the E198V variant frequency in β -tubulin isotype 2 genes. Moreover, they noted that this mutation in isotype 2 may mediate a higher level of resistance than those conferred by SNPs in isotype 1 (e.g., F200Y). However, it is important to note that there are differences in expression levels of different nematode β -tubulin isotypes based on life stage and cell type.

In silico docking of the wild-type and mutated β -tubulin isotype 1 proteins with various benzimidazole drug ligands in this study revealed that resistance-associated mutations alter binding affinities and positions. Our results showed that E198K and Q134H mutations caused a marked decrease in binding affinity, regardless of the benzimidazole ligand used. The Q134H mutation was recently found to be widespread among *A. catus* in the United States. The results of our study suggest that the same mutation may also confer resistance in *A. ceylanicus*, *A. shoshoni*, and *N. americanus*. The E198K mutation, which caused a marked reduction in binding affinities in all hookworm species studied herein, has only been reported recently in *A. suum* from Australia. The same SNP was recently reported in *H. contortus* infecting goats from several districts of Uganda. Fungal pathogens of crops in China that were resistant to benzimidazole derivatives also carry these mutations. Currently, there is no information regarding their effect on resistance among parasitic nematodes of public health concern. Hence, for the first time, we provide evidence of marked binding affinity reduction caused by the E198K mutation in hookworm β -tubulin isotype 1 proteins with several benzimidazole drugs. Moreover, we also showed that the Q134H mutation may confer resistance in many hookworm species, as proven previously in *A. catus*.

In the present study, we observed that mutations in the β -tubulin isotype 1 protein caused a shift in the ligand-binding position, modifications in bonds formed, and loss of important interactions. These alterations have been reported in previous *in silico* docking studies of other nematode species. Despite the modifications in binding positions in mutated proteins, the profile of interacting amino acids with the benzimidazole drug ligands in this study remained similar to those of the wild-type proteins. Numerous drug interactions with leucine at position 253, methionine at position 257, and alanine at position 214 have also been noted in previous prediction and molecular dynamics studies. Likewise, the frequent interactions with amino acids at 198, both in wild-type and mutated β -tubulin, observed in this study were also confirmed by prior research, thereby implicating that SNPs occurring at this position may be crucial in inducing resistance. Our results, together with those previously reported, indicate that amino acid substitutions at positions 198 and 134 caused by resistance-related SNPs induce modifications in bond formation and other ligand-protein interactions that play roles in benzimidazole resistance in helminths of public and veterinary health significance. This should be explored further in future laboratory and field studies.

The molecular dynamics simulations revealed that hookworm β -tubulins, both wild-type and mutated, bound with benzimidazole ligands.

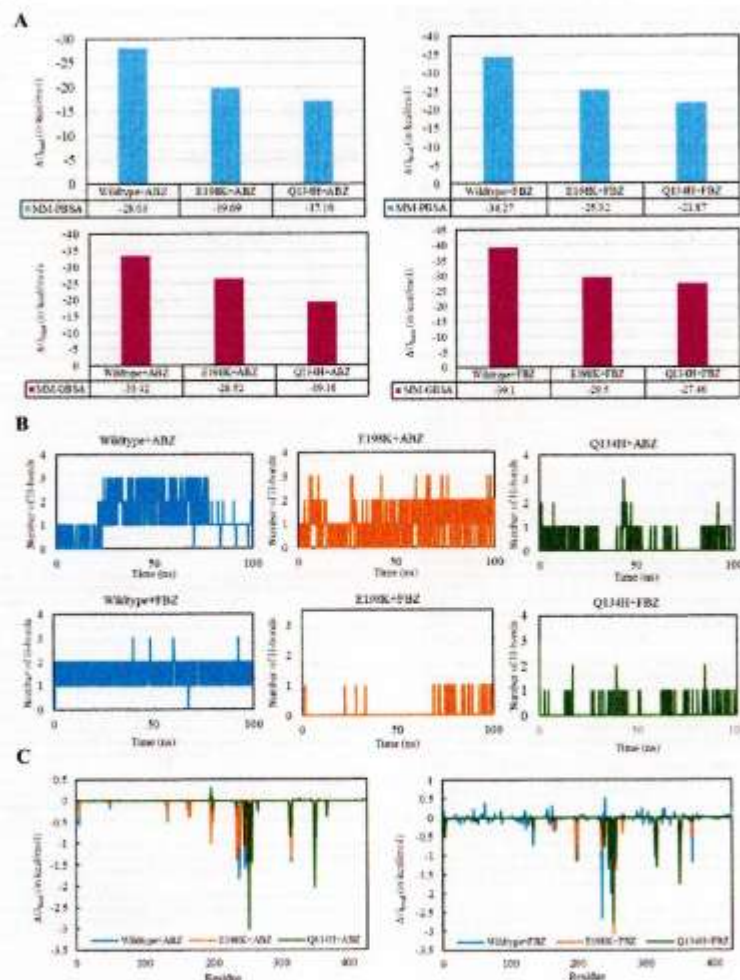


Fig. 6. The interaction of the β -tubulin-BZ complexes. **A:** The result of the MM-PBSA (Molecular Mechanics-Poisson-Boltzmann Surface Area) and MM-GBSA (Molecular Mechanics-Generalized Born Surface Area) binding free energies of the β -tubulin-BZ complexes from the MD simulation. Overall, complexes with mutated β -tubulin caused a reduction in the binding energy, resulting in weaker interactions. **B:** The hydrogen bond contact plots during the 100 ns MD simulation. The E198K and Q134H mutation in hookworm β -tubulin caused reductions in the number of hydrogen bonds formed. **C:** The per-residue energy decomposition of the binding free energy plots. It showed that the interacting amino acids involved those in residues where mutations occurred (positions 134, 167, 198, and 202). Also, the strongest interactions occurred in residues 236 to 248, 251 to 256, 315 to 318, 351, and 356. Molecular dynamics (MD) simulation analysis revealed that BZ resistance mutations reduce the binding free energy of the BZ- β -tubulin interaction. This reduction is caused by a decrease in hydrogen bond contacts. Other residues within the binding pocket that interact with the drug may also be targets of resistance mutations.

show similar behavior in terms of atomic displacements and protein backbone flexibility. This indicated that resistance-associated mutations significantly altered the simulated behavior of the β -tubulin-benzimidazole ligand complex. This observation was also noted by Jones et al.¹⁶ in dynamics simulations of *A. haemorrhoides* β -tubulin with the canonical mutations (i.e., F167Y, E198A, and F200Y) bound with ARZ. Despite these dynamic similarities, analysis of the H-bonds formed between the receptor and the benzimidazole ligand provided insights into how the E198K and Q134H mutations cause resistance. The two mutations decreased the number of H-bonds between the complex. Benzimidazole resistance-associated mutations causing reduction in interactions have also been reported in *in silico* studies that assessed *H. contortis* and *Gyrodactylus* β -tubulin, where residues cause intra-protein interactions instead of the ligand.¹⁷ The marked differences in absolute binding free energy between complexes with wild-type and mutated β -tubulin also confirm the effects of lowering the number of H-bonds observed in this study. The reduced number of hydrogen bonds resulting in diminished binding free energy is due to the loss of attraction forces and structural stability, as observed in the analysis of different drug candidates docked with the hACE2-S protein.¹⁸ Our molecular dynamics results provide an *in silico* background of how these mutations cause phenotypic resistance. Using C. elegans that were edited to have the Q134H mutation, which is widespread among A. caeni from the US, phenotypic resistance as measured by eggpress animal length was proven after exposure to 30 μ M of ARZ.¹⁹ Moreover, phenotypic resistance was also observed in C. elegans edited to contain E198K mutation when challenged with 30 μ M of ARZ and FBZ.²⁰ The fitness costs and other untoward effects of these mutations on tubulin dimerization have also been documented previously.²¹ Altogether, the molecular dynamics analysis of the β -tubulin-benzimidazole ligand complexes proved that the E198K and Q134H confer benzimidazole resistance by negatively affecting binding efficiency by reducing the number of interactions which drives down the absolute binding free energy.

5. Conclusion

Our results provided several insights into benzimidazole resistance in hookworms. Our phylogenetic analysis revealed that the hookworm β -tubulin clustered with respect to their isotypes. Hence, resistance-causing mutations in a particular β -tubulin isotype in one species may potentially cause the same as in other closely related species. Moreover, our *in silico* docking studies found that various amino acid substitutions brought about by resistance-related SNPs caused alterations in binding affinities and positions. In particular, the E198K and Q134H mutations caused marked reductions in binding affinities regardless of the hookworm species and interacting benzimidazole ligand. These mutations also resulted in shifts in the ligand-binding position, loss of interactions, and/or changes in the types of bonds formed. Molecular dynamics (MD) simulation analysis revealed that BZ resistance mutations reduce the binding free energy of the BZ- β -tubulin interaction. This reduction is caused by a decrease in hydrogen bond contacts. Additionally, other residues within the binding pocket that interact with the drug may also be targets of resistance mutations. Our results support the notion that these SNPs occurring in the β -tubulin isotype 1 gene are implicated in causing benzimidazole resistance in hookworms.

6. Ethical standards

This research does not require ethical approval for it did not use animal or human subjects.

8. Financial support

JCBT's and AEK's PhD studies are funded by the Postgraduate Scholarship for International Students of the Faculty of Medicine, Khon Kaen University, Khon Kaen University Active Recruitment Scholarships

support MPH's MSc study. SS is supported by the Fundamental Fund of Khon Kaen University and the National Science, Research, and Innovation Fund.

CRediT authorship contribution statement

Jan Glyden B. Tenorio: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Conceptualization, Muhammad Fikri Haikal: Writing – review & editing, Formal analysis, Alok Kaffar: Writing – review & editing, Formal analysis, Mark Andrian B. Maculaladi: Writing – review & editing, Formal analysis, Fredzoozer L. Orooco: Writing – review & editing, Formal analysis, Praetee Salchana: Writing – review & editing, Supervision, Conceptualization, Suttas Suttiprapa: Writing – review & editing, Supervision, Conceptualization.

7. Data availability

Data generated in this study can be made available upon request from the corresponding author.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to acknowledge Asst. Prof. Penpong Mahachaphun, PhD for sharing his expertise on molecular modeling and for providing crucial insights regarding the analysis of the molecular dynamics data.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jglr.2021.109472>.

References

- World Health Organization. Self-reported hookworm infections. <https://www.who.int/diseases/haematozoa/hookworm-infections>. Published 2021. Accessed September 16, 2021.
- Tenorio J, Pet rosopoulos and Sotgiu A. A continuing need for global worming. *Emerg Infect Dis*. 2012;18(10):1611–1612.
- Clemens AG, Alvar EA. Global distribution of human hookworm species and differences in their morbidity effects: a systematic review. *PLoS One*. 2012;7(11):e48794.
- Collica V, Bradley R, Trach B. *Ascaris lumbricoide* infections. *Emerg Infect Dis*. 2012;18(10):1611–1612.
- Kim HJ, Alvar EA, Alvar EA, et al. Global, regional, and national distribution of hookworm infections (DAATs) for 193 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet*. 2018;392(10158):1839–1852. [https://doi.org/10.1016/S0140-6736\(18\)30222-3](https://doi.org/10.1016/S0140-6736(18)30222-3).
- American PM, Lamberton PE, Fournier A, Adjuik A. Self-reported helminth infections. *Lancet*. 2018;391(10117):259–266. [https://doi.org/10.1016/S0140-6736\(18\)30222-3](https://doi.org/10.1016/S0140-6736(18)30222-3).
- Janan S, Alvar EA, Alvar EA, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet*. 2018;392(10159):1788–1858. [https://doi.org/10.1016/S0140-6736\(18\)30484-8](https://doi.org/10.1016/S0140-6736(18)30484-8).
- Strocker E, Jia AB, Trach BJ. Zoonotic *Ascaris* infections: An Update of a Continually Neglected Zoonosis. *Am J Trop Med Hyg*. 2020;100(1):54–60. <https://doi.org/10.4269/ajtmh.19-0303>.
- Zhou M, Niu H, Wang J, Shao X, et al. Insights into hookworm persistence in Asia: a systematic review and meta-analysis. *Trans R Soc Trop Med Hyg*. 2020;114(3):143–154. <https://doi.org/10.1093/trstmh/trz129>.
- Murphy MJ, Tully JI, Williams GM, et al. The control of self-reported hookworms in the Philippines: the study protocol. *BMC Public Health*. 2021;21(1):1–15. <https://doi.org/10.1186/s12889-021-10000-0>.
- World Health Organization. Guidelines for Parasitic Chemotherapy to Control Self-Transmitted Intestinal Infections in at-Risk Population Groups. Geneva: World Health Organization; 2014.

- Health Organization, 2017. <https://apps.who.int/iris/handle/10665/338961>. Accessed August 24, 2023.
- ToxCAF: Guidelines for the Diagnosis, Treatment and Control of Guinea Endoparasites in the Tropics, 2nd ed., 2019. https://www.toccaf.com/2019new/wp-content/uploads/2019/05/ToxCAF_Guinea_Book_090609es_English_Ver2.pdf. Accessed August 23, 2023.
 - ToxCAF: Guidelines for the Diagnosis, Treatment and Control of Feline Endoparasites in the Tropics, 2nd ed., 2019. https://www.toccaf.com/2019new/wp-content/uploads/2019/05/ToxCAF_Feline_Book_090609es_English_Ver2.pdf. Accessed September 13, 2023.
 - Muhammad A, Mupfema D, Mubhalla A, et al. The global progress of anti-parasitic resistance control in 2022 and World Health Organization targets for 2030. *Acta Trop* 2023;234:106900. doi:10.1016/j.actatropica.2023.106900.
 - Ukuku SM. Parasitic chemotherapy and anthelmintic resistance of soil-transmitted helminths – Can we learn anything from veterinary medicine? *One Health* 2020;10:2486. <https://doi.org/10.1016/j.onehlt.2020.10.024>.
 - Torres JCB, Tello R, Iqbal MS, Iqbal MS, et al. Anthelmintic resistance and other genetic markers associated with the *hsp70* gene in the *Ascaris suum* population. *Acta Trop* 2023;234:106901. doi:10.1016/j.actatropica.2023.106901.
 - Torres JCB, McCarroll VJ, Peralta LFV, Subirana F. Can Benzimidazole Resistance Underlie the Polyploid Increase in Controlling and Eliminating Soil-Transmitted Helminth Infections? A Meta-Analysis. *Infectious Diseases* 2024;10:1037-1043. doi:10.1007/s40127-024-00000-9.
 - van Soestem H, Peralta LFV, Subirana F, et al. Single nucleotide polymorphism (SNP) analysis for benzimidazole resistance in nematode nematodes. *Parasitology* 2021;149(10):1077-1086. <https://doi.org/10.1017/S0022278X21000076>.
 - Richard KB. Markers for benzimidazole resistance in human parasite nematodes. *Parasitology* 2007;137(10):1087-1093. <https://doi.org/10.1017/S0022278X07004102>.
 - Peralta LFV, de Fátima Bello ACP, Balboa EDS. Benzimidazole resistance in helminths: From problem to diagnosis. *Acta Trop* 2016;162:95-102. <https://doi.org/10.1016/j.actatropica.2016.06.004>.
 - Venkatesan A, Ganes P, Murugan A, et al. Molecular evidence of widespread benzimidazole drug resistance in *Ascaris suum* isolates from human dogs throughout the USA and discovery of a novel β -tubulin benzimidazole resistance mutation. *PLoS Pathog* 2021;17(3):e1008116. <https://doi.org/10.1371/journal.ppat.1008116>.
 - Stevan ML, Wiese J, Peralta LF, Lanzetta CM. Emergence of multi-drug resistant nematodes: Novel detection of *Ascaris suum* anthelmintic resistance markers by Real PCR in St. John's Island, Canada. *Am J Vet Res* 2022;83(9). <https://doi.org/10.2460/ajvr.22.0009>.
 - Ganes P, Suresh K, Suresh J, et al. Multiple anthelmintic drug resistance in *Ascaris suum* (Nematoda: Ascaridae) in a laboratory breeding and housing system in Georgia, USA. *J Am Vet Med Assoc* 2023;243(11):142-147. <https://doi.org/10.2460/javma.23.0101>.
 - Benton T, Scott J, Skjerve J. Unpublished identification of *Ascaris suum* resistance and *Urocyon* nematodes in Australia and New Zealand dogs from faecal samples. *Res Vet Clin* 2023;17(12):111-115.
 - Melero CD, Peralta LFV, Miranda GS, De Sáves VJ, Dos Santos TB, Balboa EDS. Moving beyond the state of the art of understanding resistance mechanisms to benzimidazole: benchmarking old and emerging new associated SNPs. *Acta Trop* 2022;233:106523. <https://doi.org/10.1016/j.actatropica.2022.106523>.
 - Peralta LFV, de Aguiar FPN, Subirana F, et al. Anthelmintic resistance (aR) in *Ascaris suum* nematodes is not due to single-nucleotide polymorphisms (SNPs) at codon 167, 168, or 202 of the β -tubulin gene, including another resistance mechanism. *Parasitol Res* 2019;118(2):487-493. <https://doi.org/10.1007/s00436-018-06070-3>.
 - James RP, van Vliet ACM, LaGazza EJ, Brown M. In vitro binding of Benzimidazole-5-Triazinyl With Benzimidazole Pyridinyl to Gene Expression and Ontological Variation in *Ascaris suum* Nematodes. *Infection in Tropical Diseases* 2022;5. <https://www.frontiersin.org/articles/10.3389/finf.2022.844814>. Accessed October 26, 2023.
 - James RP, van Vliet ACM, LaGazza EJ, Brown M. Identification of key interactions of benzimidazole resistance-associated genes and sequences in *Ascaris suum* by molecular docking simulations. *Int J Parasitol* 2022;52(1):137-145. <https://doi.org/10.1016/j.ijpara.2021.07.004>.
 - Stevan ML, Wiese J, Balboa E, et al. ToxCAF recommendations for the diagnosis, prevention and treatment of parasitic infections in dogs and cats in the tropics. *Int Parasitol* 2020;23(1):161-167. <https://doi.org/10.1016/j.ijpara.2019.10.004>.
 - Shaw ST, Bell M, Shafiq M, Farooq F, Bhattarai M, Wernhem Parvizi – A comprehensive manuscript for helminth genomics. *Int Parasitol* 2017;21(1):3-19. <https://doi.org/10.1016/j.ijpara.2016.12.004>.
 - The LipNet Consortium. LipNet: a worldwide bank of protein knowledge. *Nucleic Acids Res* 2016;44(D1):D26-D31. <https://doi.org/10.1093/nar/gkv124>.
 - McL T. *Protein-Ligand Docking: Practical Approaches to Molecular Modeling*. Springer; 2016. doi:10.1007/978-1-4939-9730-9.
 - Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positionspecific gap penalties and weight matrix choice. *Nucleic Acids Res* 1994;22(21):4673-4682. <https://doi.org/10.1093/nar/22.21.4673>.
 - Tomaru K, Buecher G, Kanner S, MCGA11: Molecular Evolutionary Genetic Analysis Version 11. *Mol Biol Evol* 2022;39(7):2022-2027. <https://doi.org/10.1093/molbev/msab012>.
 - Alvarez T, Kopp J, Gant N, Petrick MC. 10105-10010: an automated protein docking-resolving server. *Nucleic Acids Res* 2003;31(1):2381-2385. <https://doi.org/10.1093/nar/gkg282>.
 - Perez O, Alvarez Gallego J, Martín S, et al. High-affinity ligands of the calcitonin receptor in rabbits based on a structure-guided design. *Int J Parasitol* 2014;44(14):1429-1436. <https://doi.org/10.1016/j.ijpara.2014.08.007>.
 - Beltrami AM, McFerran N, Dwyer A, Heng L, Parniswar L. A possible model of benzimidazole binding to β -tubulin: Evidence by docking of benzimidazole derivatives. *J Mol Graph Model* 2004;23(3):275-284. <https://doi.org/10.1016/j.jmgm.2004.05.005>.
 - Ng'wira AJ, Anwar H, Sun F, Kwon YS. Anthelmintic resistance in soil-transmitted helminths: Genetically mediated. *Parasitol Res* 2022;123(1):62. <https://doi.org/10.1007/s00436-021-07274-4>.
 - Torres JCB, Balboa E, Kalle A, Subirana F, Subirana F. Benzimidazole Resistance Associated Mutations in the β -Tubulin Gene of *Haemonchus contortus*. *Parasitol Res* 2024;123(1):62. <https://doi.org/10.1007/s00436-023-04494-2>.
 - Tsai H, Chen AJ, AusDink V. Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multiple ligand support. *J Comput Chem* 2013;34(2):25-34. <https://doi.org/10.1002/jcc.23172>.
 - Kim S, Chae SA, Buhm US, et al. P-0308: Substrate and Compound Analysis. *Nucleic Acids Res* 2012;40(1):11368-11373. <https://doi.org/10.1093/nar/gkr667>.
 - Agayo-Orta B, Mirón-Luño D, Somo-Morales A, et al. Molecular basis for benzimidazole resistance from a novel β -tubulin binding site model. *J Mol Graph Model* 2013;40:28-37. <https://doi.org/10.1016/j.jmgm.2012.12.004>.
 - Davis AP, Jorgensen JL, Bourne PE, et al. Genetic landscape of drug resistance reveals evolution of anthelmintic resistance. *Cell Rep* 2022;41(2). <https://doi.org/10.1016/j.celrep.2022.111721>.
 - Sanchez G, Wainwright J, Birch S, et al. Characterization and comparative analysis of the ryanthine *Haemonchus contortus* β -tubulin gene family and implications for benzimidazole resistance in strongid nematodes. *Int J Parasitol* 2012;42(1):45-52. <https://doi.org/10.1016/j.ijpara.2011.12.011>.
 - Kalle A, Vudilo J, Naranjo A, et al. Prevalence of genotyped resistant and molecular identification of benzimidazole resistance associated with benzimidazole resistance in *Haemonchus contortus* in goats from selected districts of Uganda. *Veterinary Parasitol* 2023;302:100989. <https://doi.org/10.1016/j.vetpar.2023.100989>.
 - Lin Y, Chen B, Jiang J, Han J, Yu Y, Ma Z. Distribution and dynamics of different carbendathion resistance conferring protein variants of *Glyphosate* was collected from infested wheat fields and rice stubble in China. *Fitoplant* 2014;70(1):128-136. <https://doi.org/10.1007/s11365-013-0305-2>.
 - Agayo-Orta B, Mirón-Luño D, Medina-Franco J, et al. Towards the identification of the binding site of benzimidazole in β -tubulin of *Trichostrongylus axei* larvae from computational and experimental data. *J Mol Graph Model* 2013;41:13-23. <https://doi.org/10.1016/j.jmgm.2012.12.004>.
 - Pavlov P, Nave J, Chatterjee A, et al. Targeting SARS-CoV-2 spike protein of COVID-19 with natural occurring phytochemicals as in silico study for drug development. *J Biomed Res* 2021;35(1):626-636. <https://doi.org/10.1007/s12010-020-01070-7>.
 - Ding CM, Kuo YJ, Buchanan CM, Anderson GC. Newly identified genetic resistance to benzimidazole (BZ) anthelmintic in benzimidazole. *Int J Parasitol* 2022;52(1):149-159. <https://doi.org/10.1016/j.ijpara.2021.07.004>.
 - Ding CM, Hsieh JK, Sheng C, Long L, McQuinn PF, Anderson GC. Quantitative benzimidazole resistance and fitness effects of genetic resistance to benzimidazole. *Int J Parasitol* 2023;53(1):14-23. <https://doi.org/10.1016/j.ijpara.2022.10.004>.
 - Torres JCB, Balboa E, Kalle A, Subirana F, Subirana F. Benzimidazole resistance associated mutations improve the in silico effectiveness of benzimidazole as additional resistance mechanisms. *Int Parasitol* 2024;54(1):2730-2746. <https://doi.org/10.1016/j.ijpara.2023.10.004>.

Molecular docking and dynamics as a tool to study benzimidazole resistance in helminths: A scoping review

Jan Clyden Tenorio

Jcbtenor@louisiana.edu, jh

University of Southern Mississippi

Systematic Review

Keywords: Drug resistance, computational biology, docking, helminths

Posted Date: November 19th, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-5476123/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Additional Declarations: The authors declare no competing interests.

Abstract

Background: Benzimidazole (BZ) resistance remains an emerging grave concern in helminths of public and veterinary health concerns. Resistance against BZ drugs is due to mutations that change the amino acid comprising the β -tubulin protein, which negatively affects its interactions with BZ drug molecules. Several *in silico* modeling studies have been published to decipher the precise mechanism of BZ resistance, but inconsistencies on the resistance consequence mutations confer and the effect of different BZ ligands have led to further confusion regarding the exact mechanism of resistance. Hence, this scoping review was done to unravel the mechanism of BZ resistance based on published research on molecular docking and dynamics.

Methods: A scoping review was conducted in ScienceDirect, MEDLINE via PubMed and Scopus using the search terms "Benzimidazole Resistance AND Beta Tubulin AND Molecular Docking". A total of 37 hits were recovered and from these 6 were included after selection, inclusion, and risk of bias assessment.

Results: The six research papers included in this review studied several helminth species: *Haemonchus contortus*, *Trichostrongylus axei*, *Ancylostoma duodenale*, *Ancylostoma ceylanicum*, *Ancylostoma braziliense*, *Necator americanus*, *Trichostrongylus colubriformis*, *Trichostrongylus axei*, *Aspicularis similes*, *Ascaris suum*, *Ascaris galli*, *Parascaris equorum*, *Toxocara canis*, and *Fasciola hepatica*. The benzimidazole resistance associated mutations studied included F167Y (TTC, TTT → TAC, TAT), E198A (GAG, GAA → GCG, GCA), and F200Y (TTC, TTT → TAC, TAT). The results show that the E198A can markedly reduce the binding affinity of BZ ligand- β -tubulin interactions. The F167Y and F200Y also showed a similar effect that could vary based on the helminth species. The F200Y mutation can alter the conformation of the β -tubulin active site, negatively affecting drug binding.

Conclusion: While the impact of these mutations can vary depending on the specific helminth species and the BZ drug involved, the overall findings highlight the importance of targeting these residues for the development of novel anthelmintic strategies to address emerging drug resistance.

Introduction

Benzimidazole resistance continues to be an emerging grave concern in helminths of public and veterinary health concern. Benzimidazoles (BZ) are disruptors of microtubule polymerization by binding in the β subunit of the tubulin dimer (Whitaker et al., 2017). The binding of BZ drug molecules in the β -tubulin prevents the polymerization of tubulin subunits into microtubules, disrupting the formation of the cytoskeleton (Farrado et al., 2016). Benzimidazole drugs, like albendazole, mebendazole, and fenbendazole, are used for clinical treatment and preventive chemotherapy in humans and animals (TroCCAS, 2019; World Health Organization, 2011). Resistance against this drug class became a huge concern in the veterinary field as widespread reports of resistant livestock helminths, like *Haemonchus contortus*, *Trichostrongylus axei*, and *Trichostrongylus colubriformis* (Van Samson-Himmelstjerna et al., 2007). Among helminths of public health concern, soil-transmitted helminth infections that do not respond to conventional BZ treatment have been reported in several areas globally (Ngichich et al., 2023; Scheenkerbecher et al., 2007). Recently, the emergence of BZ-resistant helminth infections among pets (e.g., canine hookworm) in the United States and Canada raises the zoonotic threat these treatment-responsive isolates pose (Jimenez Castro et al., 2021; Tenorio et al., 2024; Veikatesan et al., 2023).

The resistance against BZ drugs is due to mutations that change the amino acid comprising the β -tubulin protein expressed by the helminth (Farrado et al., 2016). These amino acid substitutions are brought about by Single Nucleotide Polymorphisms (SNPs) (Van Samson-Himmelstjerna et al., 2007). These mutations include those that occur in amino acid positions 167 (Phenylalanine, F, TTC, TTT → Tyrosine, Y, TAC, TAT), 198 (Glutamic acid, E, GAG, GAA → Alanine, A, GCG, GCA) and 200 (Phenylalanine, F, TTC, TTT → Tyrosine, Y, TAC, TAT) (Farrado et al., 2016; Tenorio, 2022; Tenorio et al., 2024). These mutations alter the amino acid constitution of the exposed amino negatively affecting the binding of BZ drug molecules structurally or biochemically (Lacey and GR, 1994). These mutations have been reported in a variety of worms that threaten humans and animals globally (Ngichich et al., 2023). The atomic underpinnings of BZ resistance in helminths remain understudied, hence its precise mechanism has been put into question (Van Samson-Himmelstjerna et al., 2007).

Several *in silico* modeling studies utilizing advances in computational biology have been undertaken to decipher the precise mechanism of BZ resistance. These research have included modeling the wild-type protein's interaction with BZ drug ligands (Aguayo-Ortiz et al., 2013) and predicting the effects of BZ resistance mutations (Jones et al., 2022). However, inconsistency regarding the resistance effects each mutation confers and the consequences of adding numerous BZ derivatives as ligands has led to further confusion regarding the exact mechanism of resistance. Hence, this scoping review was done to unravel the mechanism of BZ resistance based on published research that used molecular docking and dynamics. The results show that the E198A can drive down the binding affinity of BZ ligand- β -tubulin interactions regardless of the species and drug used. The F167Y and F200Y also showed a similar effect that could vary based on the helminth species.

Materials and Methods

Research Questions

The scoping review was done based on the guidelines reported by the PRISMA-SuR (PRISMA Extension for Scoping Reviews) (Tricco et al., 2018) (<https://www.prisma-statement.org/scoping>). Based on published molecular docking and dynamics studies, this research aims to determine the mechanism of benzimidazole. Specifically, this research answers the following questions:

1. What are the *in silico* underpinnings of benzimidazole resistance based on molecular docking and dynamics study?
2. What are the consequences of these mutations on the measurement of binding efficiency of the β -tubulin-benzimidazole drug complex?
3. What are the consequences of these mutations on the interactions between the β -tubulin and the benzimidazole drug ligand's?

Search Strategy

A systematic search was done in three research databases: Scopus (<https://www.scopus.com/search>), ScienceDirect (<https://www.sciencedirect.com/>), and MEDLINE via PubMed (<https://pubmed.ncbi.nlm.nih.gov/>) were searched using the search term: "Benzimidazole Derivatives AND Beta Tubulin AND Molecular Docking." The literature search was done on 17 September 2024. The .csv file of the search results was downloaded.

Study Selection, Strategy, and Eligibility

Using the Mendeley citation manager (<https://www.mendeley.com/>), the .csv files were uploaded and utilized for the selection and eligibility assessment. First, duplicates and records with no titles and abstracts (e.g., indexes) were removed. Second, an initial evaluation based on the title and abstract was done. Full-length articles of the studies were accessed for further eligibility appraisal. Figure 1 summarizes the systematic literature search, selection, and eligibility evaluation done.

A study was considered eligible for selection if it fulfilled any of the following inclusion criteria:

1. Studies that utilized molecular docking in assessing the in silico effects of the BZ resistance mutations;
2. Studies that utilized molecular docking in assessing the in vivo effects of the BZ resistance mutations.

From the included studies, papers that did not meet the following criteria were excluded:

1. Studies that did not report docking scoring functions (i.e., binding affinities) and/or binding free energies (e.g., MM-PBSA or MM-GBSA);
 2. Studies that did not report the effects of BZ resistance mutations on the interaction between β -tubulin and benzimidazole drug ligand's;
 3. Studies done using non-helminth β -tubulins as macromolecules;
 4. Studies that did not use commercially available benzimidazole drugs as ligands; and
 5. Studies that utilized newly designed and synthesized benzimidazole derivatives.
6. Research not in the English language.

Risk of Bias Assessment

Due to the *in silico* and computational nature of the studies being reviewed, traditional checklists for laboratory experiments are not well-suited as the method of bias assessment. Hence, we developed a simple checklist that is based on the quality of the modeled β -tubulin macromolecule, ligand preparation, docking software, simulation quality, and data analysis utilized. The tool is in the form of a 15-item, closed-ended questionnaire (Supplementary Table 1). All included studies were evaluated using this tool.

Data Acquisition and Synthesis

The author's name, year of publication, software used in molecular docking and/or system used in molecular dynamics, helminth species of the β -tubulin used as the macromolecule, benzimidazole ligand used, BZ resistance mutation evaluated, docking scoring functions and/or binding free energies of the complex, description of the changes in interactions, and relative resistance-associated effects were the data acquired from the selected studies. Simple descriptive statistics, like counts and frequencies, were used to describe and synthesize the results of this scoping review.

Results

Characteristics of the studies included

A total of 37 hits were found in the three databases searched (Fig. 1). Fifteen of these were removed due to duplication. The full text of one article was not accessed. After the eligibility screening, two were removed for not reporting docking scoring function, eight were removed for not using helminth β -tubulin, three were dropped for using newly designed benzimidazole ligands, and one did not report the effects of the docked complexes. In total, six research papers were included in this scoping review. The six research papers included in this review studied several helminth species: *Haemonchus contortus*, *Trichostrongylus axei*, *Angiostrongylus vasorum*, *Amphistomum canaliculatum*, *Angiostrongylus ceylanicus*, *Mesocricetus auratus*, *Trichostrongylus axei*, *Trichostrongylus axei*, *Ascaris suum*, *Ascaris galli*, *Parascaris equorum*, *Fasciola canis*, and *Fasciola hepatica*. The benzimidazole resistance-associated mutations studied included F167Y (TTG, TTT → TAC, TAT), E198A (GAG, GAA → GCG, GCA), and F200Y (TTG, TTT → TAC, TAT).

In silico effects of BZ resistance-associated mutations

Table 1 summarizes computational studies investigating the molecular mechanism underlying benzimidazole (BZ) drug resistance in various helminth species. The results highlight the crucial role of specific amino acid residues (e.g., F167, E198, F200) in BZ binding and the potential mechanisms through which mutations in these residues can confer resistance. Mutations in specific amino acid residues (F167, E198, F200) within β -tubulin proteins are frequently associated with BZ drug resistance. These mutations can disrupt the conformation of the β -tubulin active site, decrease BZ binding, and reduce drug efficacy. Moreover, mutations, particularly at position 198, can lead to the loss of hydrogen bonding interactions between BZ drugs and β -tubulin, contributing to resistance. Further mutations at positions 167 and 200 may interfere with the opening of the binding site or the internalization of BZ ligands.

The impact of mutations on resistance can vary across different helminth species. For example, mutations in F167Y may have a greater impact on strongyle parasites compared to other soil-transmitted helminths. Overall, the results suggest that BZ resistance is primarily attributed to alterations in the β -tubulin binding site, hindering the effective interaction between BZ drugs and their target protein.

STUDY CODE	AUTHORS	YEAR PUBLISHED	MOLECULAR DOCKING SOFTWARE	MOLECULAR DYNAMICS SYSTEM	HELMINTH SPECIES	β -TUBULIN EVALUATED	BZ LIGAND USED	DOCKING SCORING FUNCTION(Kcal/mol)	BINDING FREE ENERGY (Kcal)
S5	Devesh-Facetti et al. (2023)	2023	AutoDock Vina program in PyMOL software	N/A	Trichostrongylus axei	Wild-type	Albendazole	-8.50	N/A
						Wild-type isotype 1	Triclabendazole	-6.87	
						Wild-type isotype 2	Triclabendazole	-4.40	
						Wild-type isotype 3	Triclabendazole	-6.38	
						Wild-type isotype 4	Triclabendazole	-6.33	
						Wild-type isotype 5	Triclabendazole	-6.17	
S6	Yashina et al. (2024)	2024	AutoDock Tools in MGL tools 1.5.6	N/A	Haemonchus contortus	Wild-type	Albendazole	-9.51	N/A

Effects of Mutations on Docking Scoring Function and Binding Free Energy

Based on the results of the included studies, the BZ resistance mutations often lead to a decrease in the docking scoring function and binding free energy, indicating a weaker interaction between the BZ drug and the mutated β -tubulin protein. However, the magnitude of the effect can vary depending on the specific mutation and the BZ drug involved. While F167Y mutations can sometimes decrease binding affinity, their effect is often less pronounced compared to mutations at other residues. Also, the impact of F167Y may vary across different helminth species. Meanwhile, the E198A mutation consistently leads to a marked decrease in binding affinity, suggesting a crucial role of this residue in BZ binding. The E198A mutation likely disrupts hydrogen bonding interactions between the BZ drug and β -tubulin. The F200Y mutations generally result in a moderate decrease in binding affinity. This mutation may alter the conformation of the β -tubulin active site, affecting drug binding. The combined effects of multiple mutations can further reduce binding affinity and enhance resistance. Likewise, the impact of mutations may vary depending on the specific BZ drug being considered. Overall, the results suggest that mutations in these key residues can disrupt the structural and electrostatic interactions between BZ drugs and β -tubulin, leading to reduced binding affinity and ultimately, drug resistance.

Discussion

This scoping review was conducted with the aim of unraveling the mechanisms of BZ resistance based on published research on molecular docking and dynamics. MEDLINE via PubMed, Scopus, and Science Direct were searched. A total of six eligible studies were included, which encompassed research that utilized β -tubulin from several helminth species and numerous benzimidazole ligands. Of the BZ mutations studied, E198A showed that it can drive down

the binding affinity of BZ ligand β -tubulin interaction. The F167Y and F200Y also showed a similar effect that could vary based on the helminth species. The F200Y mutation can alter the conformation of the β -tubulin active site, negatively affecting drug binding.

The studies included in this scoping review assessed both wild type and mutated helminth β -tubulin. The three canonical BZ resistance mutations—F167Y, E198A, and F200Y—that are induced by SNPs were all evaluated (Furtado et al., 2016). These mutations have been reported in many helminth species of public and veterinary health concern from many parts of the world (Dawson et al., 2013; George et al., 2022; Kramer Castro et al., 2021). Hence, their inclusion in computational studies was warranted. However, their actual contribution to conferring resistance has been put into question (Lacey and Gill, 1994). Also, several recent evidence of alternative resistance mechanisms, such as enzymatic biotransformation of drugs using UDP-glycosyltransferases and long non-coding RNA interference (Chen et al., 2024; Demunová et al., 2022), have been reported. However, laboratory research using gene editing that encoded resistance-associated mutations in *Caenorhabditis elegans* showed their actual potential to confer resistance, as previously mentioned (Diks et al., 2021, 2025). Particularly, varying mutations that have been reported in amino acid position 198 have been reported to make edited *C. elegans* significantly more resistant to benzimidazole treatment compared to their wild-type counterpart (Diks et al., 2021). This result echoes the finding in this systematic review that the E198A mutation had a consistent negative effect on the binding of BZ drugs and helminth β -tubulin indicating their important role in conferring resistance.

This scoping review has several limitations. First, only a few accessible databases were searched, hence there might be other research not indexed in these databases that were missed. However, searching only known indexing databases, like MEDLINE via PubMed and Scopus, ensures that only quality research papers from reputable journals are included. Second, the article nature of the research targeted for this review may lead to conclusions of insufficient evidence for the conclusions that they present due to the lack of laboratory confirmation. However, advances in computational biology have assured that the predictions are of high quality and accuracy, particularly for molecular docking and dynamic studies (Santos et al., 2019; Singh et al., 2022).

Conclusion

The computational studies included in this scoping review provide valuable insights into the molecular mechanisms underlying BZ drug resistance in various helminth species. The results consistently demonstrate that mutations in specific amino acid residues within β -tubulin proteins, particularly F167, E198, and F200, play a critical role in conferring resistance. These mutations disrupt the structural and electrostatic interactions between BZ drugs and helminth β -tubulin, leading to reduced binding affinity and ultimately, drug resistance. While the impact of these mutations can vary depending on the specific helminth species and the BZ drug involved, the overall findings highlight the importance of targeting these residues for the development of novel anthelmintic strategies to address emerging drug resistance. Future studies could explore additional mutations, investigate the interactions between BZ drugs and other β -tubulin isoforms, or investigate the potential for combination therapies to overcome resistance.

Declarations

Supplementary Materials

Supplementary tables and figures are available at [30].

Acknowledgments

None.

Funding

No funding was received to assist with the preparation of this manuscript.

Conflict of Interests

The author declares that there is no conflict of any financial or non-financial interests regarding the publication of this paper.

Ethics Approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Data Availability

Not applicable.

References

Table 1
The key results of the studies included in the scoping review.

STUDY CODE	AUTHORS	YEAR PUBLISHED	MOLECULAR DOCKING SOFTWARE	MOLECULAR DYNAMICS SYSTEM	HELMINTH SPECIES	β-TUBULIN EVALUATED	BZ LIGAND USED	DOCKING SCORING FUNCTION(Kcal/mol)	BINDING FREE ENERGY (Kcal/n)	
S1	Aguayo-Ortiz et al. (2018)	2018	AutoDock 4.2	GROMACS 4.5.3	Haemonchus contortus	Wild-type	Albendazole	-8.10	-68.41	
							Carbendazim	-6.98	-53.08	
							Oxibendazole	-6.17	-47.27	
							Parbendazole	-8.09	61.59	
							Luabendazole	9.46	-64.77	
							Mebendazole	-9.49	-66.58	
							Nicodazole	-9.36	-44.61	
							F167Y	Albendazole	-8.07	N/A
								Carbendazim	-7.62	
								Oxibendazole	-8.09	
								Parbendazole	8.15	
								Luxabendazole	4.70	
								Mebendazole	9.46	
								Nicodazole	-9.35	
								Albendazole	-7.03	
							E198A	Carbendazim	-6.37	
								Oxibendazole	-6.66	
								Parbendazole	-7.05	
								Luabendazole	8.82	
								Mebendazole	8.76	
Nicodazole	-7.99									
F200Y	Albendazole	-8.32								
	Carbendazim	-7.19								
	Oxibendazole	-8.23								
	Parbendazole	-8.26								
	Luabendazole	9.62								
	Mebendazole	-6.48								
	Nicodazole	9.33								
	Albendazole	-7.70	-62.67							
	Carbendazim	-7.05	-21.74							
	Oxibendazole	-7.41	-31.92							
	Parbendazole	-7.92	-37.29							
	Luabendazole	-9.41	-44.71							

STUDY CODE	AUTHORS	YEAR PUBLISHED	MOLECULAR DOCKING SOFTWARE	MOLECULAR DYNAMICS SYSTEM	HELMINTH SPECIES	β-TUBULIN EVALUATED	BZ LIGAND USED	DOCKING SCORING FUNCTION(Kcal/mol)	BINDING FREE ENERGY (Kcal/n)		
							Mebendazole	-6.73	-43.04		
							Nicodazole	-8.62	-34.34		
S2	Jones et al. (2022a)	2022	Autodock v12 v1.1.2	Molecular Operating Environment (MOE) version 2020.01	Ascaris suum	Wild-type	Albendazole	-8.45	N/A		
							F167Y	Albendazole	-8.04		
							E198A	Albendazole	-8.53		
S4	Jones et al. (2022b)	2022	Autodock v12 v1.1.2	Molecular Operating Environment (MOE) version 2020.01	Anchistoma duodenale	Wild-type	Albendazole	-8.55	N/A		
							F167Y	Albendazole	-8.19		
							E198A	Albendazole	-8.14		
							F200Y	Albendazole	-7.72		
							Trichostrongylus axei	Wild Type	Albendazole	-8.13	
								F167Y	Albendazole	-4.82	
								F198A	Albendazole	-7.78	
								F200Y	Albendazole	-10.48	
							Anisakis simplex	Wild-type	Albendazole	-7.74	
Ascaridia galli	Wild-type	Albendazole	-8.25								
Pseudocapillaria equorum	Wild-type	Albendazole	-8.19								
Tricostema canis	Wild-type	Albendazole	8.82								
Anchistoma caninum	Wild-type	Albendazole	-7.64								
Anchistoma cephalicum	Wild-type	Albendazole	-8.25								
Nippostrongylus brasiliensis	Wild-type	Albendazole	-7.54								

1. Aguayo-Olivero, R., Miranda-Lucio, O., Medina-Fernandez, J.L., Castiblanco, R., Yipor-Molina, L., Hernandez-Luz, F., Hernández-Camacho, A., 2013. Towards the identification of the binding site of benzimidazole to β -tubulin of *Trichostrongylus axei*: Insights from computational and experimental data. *Journal of Molecular Graphics and Modelling* 41, 12–19. <https://doi.org/10.1016/j.jmgm.2013.01.007>
2. Chen, X., Wang, T., Guo, W., Yan, Y., Xie, H., Yu, Y., Liu, C., Gao, W., Wang, W., Wang, R., 2024. Transcriptome reveals the roles and potential mechanisms of lncRNAs in the regulation of albendazole resistance in *Haemonchus contortus*. *BMC Genomics* 25, 188. <https://doi.org/10.1186/s12864-024-10096-6>
3. Davies, A., Halperin, C.M., Charrier, T.S., Meandozojo, C., Kilani, J., Kaplan, R.M., Szeg, T.G., Itaghdou, Y., Scott, M.E., Bakst, M.-O., Pritchard, R.K., 2013. Association between Response to Albendazole Treatment and β -Tubulin Genotype Frequencies in Soil-Transmitted Helminths. *PLoS Negl Trop Dis* 7, e2247. <https://doi.org/10.1371/journal.pntd.002247>
4. Dika, C.M., Rahmat, S.R., Sheng, Q., Long, L., McGrath, P.T., Anderson, E.C., 2020. Quantitative benzimidazole resistance and fitness effects of parasite: nematode β -tubulin alleles. *Int J Parasitol Drugs Drug Resist* 14, 29–36. <https://doi.org/10.1016/j.ijpdr.2020.08.003>
5. Dika, C.M., Koury, C.J., Buchanan, C.M., Anderson, E.C., 2021. Newly identified parasite nematode β -tubulin alleles confer resistance to benzimidazoles. *International Journal for Parasitology: Drugs and Drug Resistance* 17, 168–175. <https://doi.org/10.1016/j.ijpdr.2021.05.006>
6. Demunová, D., Navrátilová, M., Kollerová, K., Ambrož, M., Skálová, L., Matoušková, P., 2023. The induction and inhibition of UDP-glycoyltransferases in *Haemonchus contortus* and their role in the metabolism of albendazole. *International Journal for Parasitology: Drugs and Drug Resistance* 19, 56–64. <https://doi.org/10.1016/j.ijpdr.2022.06.001>
7. Furtado, L.F.V., De Paiva-Bello, A.C.P., Ribeiro, É.M.L., 2016. Benzimidazole resistance in helminths: From problem to diagnosis. *Acta Tropica* 162, 95–102. <https://doi.org/10.1016/j.actatropica.2016.05.021>
8. George, S., Basurto, P., Akari, J., Ochoa, J., Harrison, L.M., Bijušić, K., Knight, J.R., Humphries, D., Wilson, M.O., Coccone, A., Coppola, M., 2023. Application of multiple amplicon deep-sequencing (MAD-seq) to screen for putative drug resistance markers in the Neotropical nematode *scyphi-1 β -tubulin gene. *Sci Rep* 13, 11439. <https://doi.org/10.1038/s41598-023-15719-1>*
9. Jimenez-Castro, P.D., Venkatesan, A., Redman, E., Chen, R., Malanika, A., Huff, H., Zukhree Saleem, D.A., Arsenenko, B., Gilman, J.S., Kaplan, R.M., 2021. Multiple drug resistance in hookworms infecting greyhound dogs in the USA. *International Journal for Parasitology: Drugs and Drug Resistance* 17, 107–117. <https://doi.org/10.1016/j.ijpdr.2021.08.005>
10. Jones, R.P., van Vleet, A.H.M., LaCourse, E.J., Betson, M., 2022. Identification of key interactions of benzimidazole resistance-associated amino acid mutations in *Ancylostoma* β -tubulins by molecular docking simulations. *Sci Rep* 12, 13725. <https://doi.org/10.1038/s41598-022-16765-4>
11. Lacey, E., Gill, J.H., 1994. Biochemistry of benzimidazole resistance. *Acta Trop* 56, 245–262. [https://doi.org/10.1016/0001-706x\(94\)90066-3](https://doi.org/10.1016/0001-706x(94)90066-3)
12. Mjčić, A.I., Amjad, I.D., Bui, F., Kumar, S., 2023. Anthelmintic resistance in soil-transmitted helminths: One Health considerations. *Parasitol Res* 123, 62. <https://doi.org/10.1007/s00436-023-05088-8>
13. Santos, L.H.S., Ferreira, B.S., Caffarena, E.B., 2019. Integrating Molecular Docking and Molecular Dynamics Simulations. In: De Azevedo, W.F. (Ed.), *Docking Screens for Drug Discovery: Methods in Molecular Biology*. Springer New York, New York, NY, pp. 13–34. https://doi.org/10.1007/978-1-4939-9752-2_3
14. Schwenkenbecher, J.M., Alfonso, M., Bickel, O., Kaplan, R.M., 2007. Characterisation of β -tubulin genes in hookworms and investigation of resistance-associated mutations using real-time PCR. *Mol Biochem Parasitol* 156, 167–174. <https://doi.org/10.1016/j.molbiopara.2007.07.019>
15. Singh, S., Bari Bakht, G., Singh, D.N., 2022. Molecular docking and molecular dynamics simulation. In: *Bioinformatics*. Elsevier, pp. 291–304. <https://doi.org/10.1016/B978-0-323-89775-4.000144>
16. Tenorio, J.C.B., 2020. Canine hookworms in the Philippines—Very common but very much neglected in veterinary research. *Frontiers in Veterinary Science* 10.
17. Tenorio, J.C.B., Tubiao, I.R.B., Ipankavac, T., Ybañez, A.F., Tiwananibagom, S., Tangkavattana, S., Sattaprapa, S., 2024. *Ancylostoma ceylanicum* and other zoonotic canine hookworms: neglected public and animal health risks in the Asia-Pacific region. *Animal Diseases* 4, 11. <https://doi.org/10.1186/s44149-024-00211-7>
18. Tocco, A.C., Lillie, E., Zarr, W., O'Brien, K.K., Colquhoun, H., Lencic, D., Mohr, O., Peters, M.D.J., Horsley, T., Weeks, L., Hempel, S., AHLCA, Dhong, C., McGowan, J., Stewart, L., Hartling, L., Aldrich, A., Wilson, M.G., Genuis, C., Law, S., Godfrey, C.M., Macdonald, M.T., Langlois, E.V., Soares-Waike, K., Morin, J., Dillford, T., Tunçalp, O., Orosco, S.E., 2019. PRISMA Extension for Scoping Reviews (PRISMA-ScR): Checklist and Explanation. *Ann Intern Med* 170, 467–473. <https://doi.org/10.7326/M18-0850>
19. TruCCAP, 2019. Guidelines for the diagnosis, treatment and control of canine endoparasites in the tropics, 2nd ed.
20. Venkatesan, A., Castro, P.D.J., Marasutti, A., Horvath, H., Chen, R., Redman, E., Dunn, K., Collins, J.B., Fraser, J.S., Anderson, E.C., Kaplan, R.M., Gilman, J.S., 2023. Molecular evidence of widespread benzimidazole drug resistance in *Ancylostomes caninum* from domestic dogs throughout the USA and discovery of a novel β -tubulin benzimidazole resistance mutation. *PLoS Pathogens* 19, e1011146. <https://doi.org/10.1371/journal.ppat.1011146>
21. Von Samson-Himmelfarb, G., Backhaus, W.J., McCARTHY, J.S., Shuck, R.J., 2007. Single nucleotide polymorphism (SNP) markers for benzimidazole resistance in veterinary nematodes. *Parasitology* 134, 1077–1086. <https://doi.org/10.1017/S002182007000054>
22. Whitaker, J.H., Carlson, S.A., Jones, D.E., Besser, M.T., 2017. Molecular mechanisms for anthelmintic resistance in strongyle nematode parasites of veterinary importance. *J vet Pharmacol Therap* 40, 105–115. <https://doi.org/10.1111/jvp.12330>
23. World Health Organization, 2011. Helminth control in school-age children: a guide for managers of control programmes. World Health Organization.

Figures

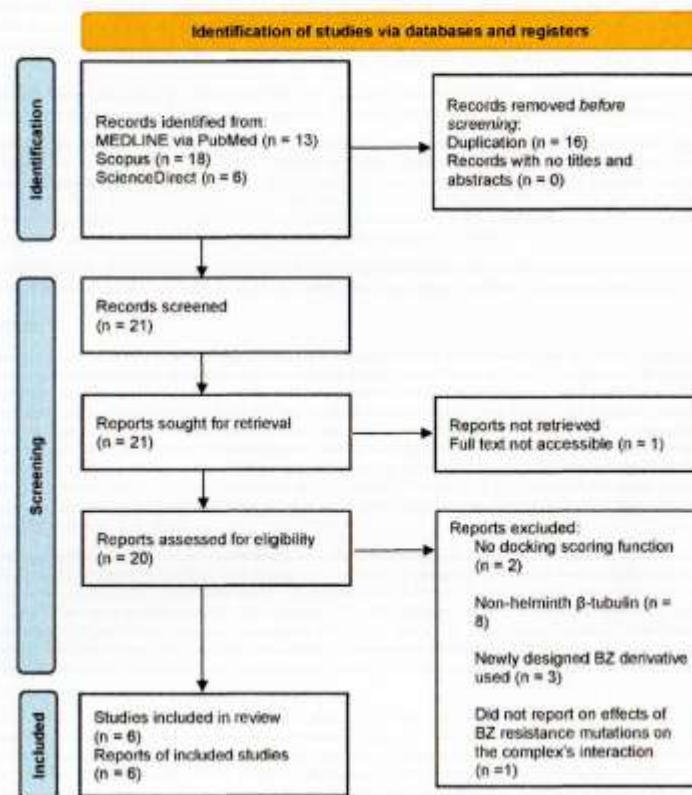


Figure 1

PRISMA-ScR (Preferred Reporting Items for Systematic Reviews and Meta-Analyses-Extension for Scoping Reviews) flowchart of the screening, selection, and eligibility selection in this study.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download:

- [SupplementaryMaterials.docx](#)



Letter to the Editor

Can resistant *Ancylostoma ceylanicum* arise, persist, and be transmitted between humans and animals?Jan Clyden B. Tenorio^{1*}¹Department of Veterinary Parasitological Sciences, College of Veterinary Medicine, University of Southern Mindanao, Davao 807, Cebu, Philippines

LETTER INFO

Letter history:

Received 17 September 2024

Received in revised form 5 November 2024

Accepted 5 November 2024

To the Editor

Ancylostoma ceylanicum is a zoonotic hookworm that is prevalent in Asia-Pacific region where it is predominant among humans and companion animals [1–3]. This hookworm is reported to affect nearly 30% to 50% of people in countries within Southeast Asia [2, 4, 5]. Among animals, particularly dogs and cats, prevalence rates are 35% but can be as high as more than 95% within the same region [5, 6]. Hence, it is now believed that this zoonotic hookworm is the number one cause of infections among animals and is second only to *Necator americanus* among humans [2, 7]. Transmission dynamics, as revealed by phylogenetic and population genetics analysis, show that there are three pathways that *A. ceylanicum* takes to proliferate between humans and animals (Figure 1). A larger group of the population circulates between humans and animals, while two smaller populations persist within both hosts [2, 6, 8]. The true zoonotic nature of this hookworm presents a serious One Health threat to human and animal populations cohabitating in endemic areas.

Control and elimination has primarily been through pharmaceutical interventions: Mass Drug Administration (MDA) with benzimidazoles in humans and routine veterinary deworming for pet animals [9–11]. Resistance to benzimidazoles, such as albendazole and fenbendazole, is caused by specific genetic variations known as Single Nucleotide Polymorphisms (SNPs) in the β -tubulin isotype 1 gene [14]. These SNPs result in amino acid substitutions in the protein, altering its structure and hindering the drug's ability to bind to its target site [12]. The F167Y and Q134H mutations have been reported in the dog hookworm, *A. caninum*, in the United States [13, 14]. Moreover, the mutations have also been reported in the human hookworm, *Necator americanus* [15]. Among *A. ceylanicum*, mutations have been found in laboratory specimens that have been induced to gain resistance against albendazole [16]. The documented emergence of drug resistance in other hookworm species, the aforementioned study of laboratory-induced resistance, and its potential for widespread distribution highlight the danger of resistance development in *A. ceylanicum*. Such resistance could significantly compromise our ability to control and eliminate this parasite, posing a serious risk to public health.

Can *A. ceylanicum* develop resistance to benzimidazole drugs, outcompete other parasite species after treatment, and be spread between animals and humans (Figure 2)? Given the potential transmission routes of zoonotic *A. ceylanicum* and the risk of drug resistance development from both

animals and humans, repeated drug treatments could lead to the emergence of resistant hookworm populations that can spread between human and animal populations. If resistant *A. ceylanicum* populations become widespread in both humans and animals, it could have severe consequences for public health and veterinary medicine. One way to determine if drug-resistant *A. ceylanicum* populations have emerged is to analyze the genetic makeup of isolates from both humans and animals using the β -tubulin isotype 1 gene as a marker. By analyzing the genetic makeup of these isolates, researchers can potentially trace the lineage and transmission routes of drug-resistant zoonotic hookworms. This analysis may reveal information about how genetic variations associated with benzimidazole resistance are transmitted between humans and animals. The occurrence of these mutations has been reported. While whole-genome sequencing could provide more comprehensive genetic information, it requires substantial resources and collaboration. In addition to genetic analysis, it will be crucial to monitor the emergence of drug resistance in both humans and animals following established guidelines from organizations like the World Health Organization and the World Association for the Advancement of Veterinary Parasitology [17–19].

In conclusion, the zoonotic nature of *A. ceylanicum* presents a significant One Health challenge due to its potential for transmission between humans and animals. The emergence of benzimidazole resistance poses a significant threat to public health and animal welfare. Population genetic analysis using the β -tubulin isotype 1 gene can be employed to track the transmission dynamics of resistant hookworms and assess their potential for zoonotic circulation. By understanding the genetic structure and transmission pathways of these resistant populations, we can develop more effective control strategies and mitigate the risks associated with benzimidazole resistance. Effective control and preventative efforts against drug-resistant *A. ceylanicum* may include combination treatment, targeted treatment to the most at-risk populations, and monitoring resistance occurrence.

In the Asia-Pacific region, most *A. ceylanicum* infections are transmitted between humans and animals (Red), with fewer isolates occurring and persisting solely in humans (Green) or animals (Orange) [2, 4, 6, 8].

Figure 1. The transmission dynamics of *Ancylostoma ceylanicum* in the Asia-Pacific region.



Figure 2. Can resistant *Ancylostoma ceylanicum* isolates arise, persist, and be transmitted between humans and animals?



Drug resistance in *A. ceylanicum* can arise from both human and animal interventions, such as mass drug administration and veterinary deworming. Due to the parasite's zoonotic nature, resistant hookworms could potentially become dominant in either humans or animals and spread between species.

Conflict of Interest

The author has no conflict of interest to declare.

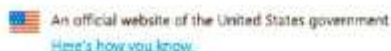
References

1. Tenorio JCB. Canine hookworms in the Philippines—Very common but very much neglected in veterinary research. *Promiers in Veterinary Science*. 2023;10. Accessed January 3, 2024. <https://www.frontiersin.org/articles/10.3389/fvets.2023.1297962>
2. Tenorio JCB, Tabios IKB, Inpankaew T, et al. *Ancylostoma ceylanicum* and other zoonotic canine hookworms: neglected public and animal health risks in the Asia-Pacific region. *Animal Diseases*. 2024;4(1):11. doi:10.1186/s44149-024-09117-y

3. Colella V, Bradbury R, Traub R. *Ancylostoma ceylanicum*. *Trends in Parasitology*. 2021;37(9):844-845. doi:10.1016/j.pt.2021.04.013
4. Inpankaew T, Schür F, Dalsgaard A, et al. High Prevalence of *Ancylostoma ceylanicum* Hookworm Infections in Humans, Cambodia, 2012. *Emerg Infect Dis*. 2014;20(6):976-982. doi:10.3201/eid2006.131770
5. Ayta OP, McManus DF, Weerakoon KG, et al. Molecular identification of *Ancylostoma ceylanicum* in the Philippines. *Parasitology*. 2020;147(14):1718-1722. doi:10.1017/S0031182020001547
6. Kludkempetch D, Yangtrongsup S, Tiwananthagnara S. *Ancylostoma ceylanicum*: The Neglected Zoonotic Parasite of Community Dogs in Thailand and Its Genetic Diversity among Asian Countries. *Animals*. 2020;10(11):2154. doi:10.3390/ani10112154
7. Traub RJ, Zendejas-Heredia PA, Miasetti L, Colella V. Zoonotic hookworms of dogs and cats – lessons from the past to inform current knowledge and future directions of research. *International Journal for Parasitology*. 2021;51(13-14):1233-1241. doi:10.1016/j.ijpara.2021.10.005
8. Phoosangwalthong P, Kamyngkited K, Kengradomkij C, Chinnon W, Odermatt P, Inpankaew T. Molecular Detection and Genetic Characterization of Zoonotic Hookworm in Semi-Domesticated Cats Residing in Monasteries in Bangkok, Thailand. *Trop Med Infect Dis*. 2023;8(2):122. doi:10.3390/tropicalmed8020122
9. Matheig MLL, Tallo VL, Williams GM, et al. The control of soil-transmitted helminthiases in the Philippines: the story continues. *Infect Dis Poverty*. 2021;10(1):85. doi:10.1186/s40249-021-00870-z
10. TroCCAP. Guidelines for the Diagnosis, Treatment and Control of Canine Endoparasites in the Tropics. 2nd ed.; 2019. Accessed August 24, 2023. https://www.troccap.com/2017press/wp-content/uploads/2019/05/TroCCAP_Canine_Endo_Guidelines_English_Ver2.pdf
11. TroCCAP. Guidelines for the Diagnosis, Treatment and Control of Feline Endoparasites in the Tropics. 2nd ed.; 2019. Accessed September 15, 2023. https://www.troccap.com/2017press/wp-content/uploads/2019/06/TroCCAP_Feline_Endo_Guidelines_English_Ver2.pdf
12. Furtado LFV, De Paiva Bello ACP, Rabelo EML. Benzimidazole resistance in helminths: From problem to diagnosis. *Acta Tropica*. 2016;162:95-102. doi:10.1016/j.actatropica.2016.06.021
13. Venkatesan A, Castro PDJ, Morosetti A, et al. Molecular evidence of widespread benzimidazole drug resistance in *Ancylostoma caninum* from domestic dogs throughout the USA and discovery of a novel β -tubulin benzimidazole resistance mutation. *PLoS Pathogens*. 2023;19(3):e1011146. doi:10.1371/journal.ppat.1011146
14. Jimenez Castro PD, Howell SB, Schaefer JJ, Avramenko RW, Gilbeard JS, Kaplan RM. Multiple drug resistance in the canine hookworm *Ancylostoma caninum*: an emerging threat? *Parasites & Vectors*. 2019;12(1):576. doi:10.1186/s13071-019-3828-6
15. Grau-Pujol B, Gandasegui J, Escola V, et al. Single-Nucleotide Polymorphisms in the Beta-Tubulin Gene and Its Relationship with Treatment Response to Albendazole in Human Soil-Transmitted Helminths in Southern Mozambique. *The American Journal of Tropical Medicine and Hygiene*.

- 2022;107(3):649-657. doi:10.4269/ajtmh.21-0948
16. Medeiros CDS, Furtado LFV, Miranda GS, Da Silva VJ, Dos Santos TR, Rabelo EML. Moving beyond the state of the art of understanding resistance mechanisms in hookworms: confirming old and suggesting new associated SNPs. *Acta Tropica*. 2022;233:106533. doi:10.1016/j.actatropica.2022.106533
17. World Health Organization. Assessing the Efficacy of Anthelmintic Drugs against Schistosomiasis and Soil-Transmitted Helminthiases. World Health Organization, 2013. Accessed August 20, 2023. <https://apps.who.int/iris/handle/10665/79019>
18. Kaplan RM, Donwood MJ, Nielsen MK, et al. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) guideline for diagnosing anthelmintic resistance using the faecal egg count reduction test in ruminants, horses and swine. *Veterinary Parasitology*. 2023;318:109936. doi:10.1016/j.vetpar.2023.109936
19. Geurden T, Smith ER, Vercruyse J, Yazwinski T, Settle T, Nielsen MK. World association for the advancement of veterinary parasitology (WAAVP) guideline for the evaluation of the efficacy of anthelmintics in food-producing and companion animals: general guidelines. *Veterinary Parasitology*. 2022;304:109698. doi:10.1016/j.vetpar.2022.109698

Tenorio, J. C. Can resistant *Ancylostoma ceylanicum* arise and spread between humans and animals? *Microbes Infect Dis* 2025, 6(4): 6864-6867.



FULL-TEXT LINKS



Anat Histol Embryol. 2024 Jan;53(1):e12970. doi: 10.1111/ahe.12970. Epub 2023 Sep 23.

Ultrastructural evidence of telocytes in the embryonic chick heart

Khan Lamanero Junatas^{1, 2}, Liesbeth Couck¹, Hanna Tay¹, Fred Sinowatz³, Wim Van Den Broeck¹

Affiliations

PMID: 37740674 DOI: 10.1111/ahe.12970

Abstract

The cardiac telocyte (TC) is a novel interstitial cell type with a unique ultrastructure and great potential in therapy. The present study examined its presence in the heart of chicken embryos ageing 7–15 days old (Hamburger-Hamilton [HH] stages 31–41) using transmission electron microscopy. TCs were identified across all stages in the atrial and ventricular myocardium, close to maturing cardiomyocytes, blood vessels and lymphatics. Early-stage TCs have immature features resembling mesenchymal cells. Late-stage TCs were distinct, possessing the cytoplasmic prolongations termed telopodes (Tps), which are very long and thin, usually 1–3 in number, and display a moniliform appearance and have an average thickness below 0.2 μm . TCs residing in the epicardium and endocardium were also detected. In the subepicardium near developing coronary vessels, they were localized in the cardiac stem cell niches, coexisting with cardiac stem cells and cardiomyocyte progenitors. Electron-dense structures and the release of extracellular vesicles were observed between embryonic TCs and surrounding structures, suggesting roles in intercellular communication, cardiomyocyte differentiation and maturation, angiogenesis, and stem cell nursing and guidance.

Keywords: chick; embryo; heart; morphogenesis; telocyte; telopode.

© 2023 Wiley-VCH GmbH. Published by John Wiley & Sons Ltd.

[PubMed Disclaimer](#)

Related information

[GEO Profiles](#)

[MedGen](#)

LinkOut - more resources

[Full Text Sources](#)

[Ovid Technologies, Inc.](#)

[Wiley](#)

corrected for differences. In seasonal comparison, the values for winter were used as the calibrator, while for sex differences, the ram's values were used as the calibrator.

$$\text{Gene expression ratio} = \frac{(E_{\text{HUNG}})^{\Delta CT \text{ GOE}}}{(E_{\text{DORPER}})^{\Delta CT \text{ HUNG}}}$$

E: EGM: corrected efficiency of the gene of interest (GOI); *CT*: *CT*: corrected efficiency of the normalizing gene; *HUNG*: *CT*: GOI; *HUNG*: *CT*: cycle threshold value of the GOI; *DORPER*: *CT*: GOI; *DORPER*: *CT*: cycle threshold value of the HUNG.

Gene expression ratios means, standard deviation, and standard error were presented and graphically compared between the values for the summer and winter seasons and in-season comparison between rams and ewes. Meteorological and gene expression data were downloaded and processed using Microsoft Excel (Microsoft Corporation, Redmond, WA), including tabular and graphical presentations [22-23]. For analysis, the differences of *IL-10* gene expression between seasons (summer and winter), between the breeds, and sexes (rams and ewes) in every season were determined through Student's *t*-test [24] using the average cycle threshold (CT) values for the gene of interest. A difference with *P* < 0.05 value was considered statistically significant. Data analysis was carried out using R program for Windows [25].

3 Results

3.1. Climatological variables

The 30-month climatological records, which span from 2018 to 2023, show the seasonal variations of meteorological variables such as temperature, relative humidity, and THI surrounding the study's sample collection period. Environmental temperature and air relative humidity were factors that affected THI. The highest THI values were observed during the summer months of June, July, and August, which correspond to high-temperature levels. Conversely, relative humidity falls during periods of high temperature, while it is high during low-temperature periods such as the winter season (Figure 1).



Figure 1. 30-month climatological record in the farm location. The mean THI values during sample collection days were highlighted in red (summer) and blue (winter) bars.

The data on the climatic condition during the collection of samples in summer and winter are presented in Table 2. The summer data collection day was characteristically hot and dry, with temperature recorded at 32.80°C and relative humidity at 34.50%. In contrast, the winter data collection day was cold and humid, with temperature recorded at -3.33°C and relative humidity at 99.70%. The calculated THI during summer collection day was 79.0, indicating severe heat stress, while the THI during winter was 26.1, meaning there was no heat stress in the animals. Figure 2 shows the heat stress intensities that corresponded with the THI values during the sample collection and entire study period.

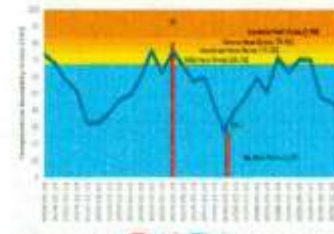


Figure 2. Heat stress levels according to THI values in the 30-month period with the THI values during the hours of sample collection, highlighted in red bars, indicating the levels of heat stress according to Lewis Ricks et al. [19].

For most of the year, THI levels were below the heat stress threshold of 65. High THI values were recorded during the summer season, indicating heat stress conditions. Mean THI levels during the summer season show moderate heat stress, but there were times during the day that were marked with elevated THI values, and thus characterized heat stress to be severe. The hour of sample collection for summer shows that the animals were subjected to severe heat stress conditions, with THI values at 79.0, several points higher than the mean THI during that day. On the contrary, the absence of heat stress was established during the winter sample collection with a THI of 26.1.

3.2. Expression of *IL-10* gene

Following qPCR analysis to determine the gene expression levels of *IL-10* in the experimental animals, relative expression was calculated using the Pfaffl method where the winter season values were used as calibrator. For all animals, mean gene expression ratio during the summer season was 16.82 with a standard deviation of 20.85 (Table 3). In contrast, the winter mean gene expression ratio was 1.18 with a standard deviation of 0.61. Relative gene expression ratios of *IL-10* in summer and winter seasons were presented in Figure 3 with winter season value as calibrator. A higher gene expression ratio of *IL-10* is found in the summer season as compared to the winter season ratio.

Table 2. Climatological data on the day of sample collection

Season	Sampling date	Sampling time	Temperature (°C)	Relative humidity (%)	THI	Heat Stress Rate
Summer	13/08/2019	12.00-13.00	32.80	34.50	79.0	Severe
Winter	22/01/2020	12.00-13.30	-3.33	99.70	26.1	No Stress

Table 3. Relative Gene Expression of *IL-10* in Hungarian Indigenous Tsigai and Dorper Sheep during summer and winter seasons

Breed	Relative gene expression (Mean ± SD)	
	Summer	Winter
Overall	16.82 ± 20.85	1.18 ± 0.61
Hungarian Indigenous Tsigai	32.3 ± 15.25	16.2 ± 18.53
White Dorper	1.1 ± 0.58	1.04 ± 0.35

SD: Standard Deviation

Breed-wise, the Hungarian Indigenous Tsigai sheep has an *IL-10* gene expression ratio mean of 32.3 and a standard deviation of 15.25 during summer. The White Dorper, on the other hand, has a mean of 16.2 and a standard deviation of 18.53 during summer. Here, the White Dorper showed a numerically higher ratio of gene expression during the summer seasons compared to the Hungarian Indigenous Tsigai.

The gene expression ratio of all the animals and the two breeds during the summer and winter are graphically presented and compared in Figure 3. The average CT-values were used to statistically analyze differences among the gene expression in 2 seasons (Table 4), between the two breeds, and among

sexes. In all animals, expression levels of *IL-10* were significantly higher (*P* < 0.00019) during the summer season than those samples collected during the winter season. Within-breed comparison of summer and winter CT-values showed significantly higher *IL-10* expression during the summer season in both breeds (Hungarian Indigenous Tsigai *P* = 0.015 & White Dorper *P* = 0.094). Between the two breeds, Hungarian Indigenous Tsigai and White Dorper, there is no significant difference in the CT-values during the summer and winter seasons. Similarly, no significant difference between ewes and rams was found during the two seasons in terms of relative gene expression ratio and CT-values (Table 5).

Table 4. Mean and standard deviation of CT-value of *IL-10* of Hungarian Indigenous Tsigai and White Dorper in summer and winter seasons.

Season	CT-value (Mean ± SD)			P-value
	Hungarian Indigenous Tsigai	White Dorper	Overall	
Summer	32.87 ± 1.84 ^a	32.62 ± 2.27 ^a	32.74 ± 1.92 ^a	0.87
Winter	29.27 ± 1.44 ^b	28.69 ± 0.54 ^b	28.66 ± 1.0 ^b	0.12
Overall	31.25 ± 2.25	30.25 ± 2.20	30.88 ± 2.75	
P-value	0.923	0.604	0.00019	

SD: Standard Deviation; Note: The different superscripts show significantly different values (*P* < 0.05).

Table 5. Relative gene expression ratio and CT-value of *IL-10* of Ewe and Ram Hungarian Tsigai and White Dorper Sheep in summer and winter seasons.

Season	Group	Hungarian Indigenous Tsigai		White Dorper	
		Relative Gene Expression Mean	CT-value Mean	Relative Gene Expression Mean	CT-value Mean
Summer	Ewe	0.01	34.205	177.61	30.765
	Ram	0.06	31.53	1.05	34.475
Winter	Ewe	0.42	36.885	0.6	30.085
	Ram	0.01	28.36	1.01	28.235

Continental vs. tropical breed: immunity comparison under heat stress conditions utilizing qRT-PCR technique

Botond Fajardó^{1,2}, Putri Kusuma Astuti^{3,4,5}, Zoltán Bági¹, Lídia Bodnag¹, Tímea Páti¹, Gabriella Skuda⁶, Husein Ullari⁷, George Wanjala^{1,4}, Szilvia Kusza⁸

¹ Centre for Agricultural Genomics and Biotechnology, University of Debrecen, 4002 Debrecen, Egervény tér 1, Hungary;

² Department of Aquaculture-Breeding of Animal Industry, 1106 Dávidov, Queson City, Philippines;

³ College of Veterinary Medicine, University of Southern Mindanao, Kabanua, Cotabato 9407 Philippines;

⁴ Doctoral School of Animal Science, University of Debrecen, 4002 Debrecen, Böszörményi út 138, Hungary;

⁵ Department of Animal Breeding and Reproduction, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia;

⁶ Department of Animal Biotechnology, Hungarian University of Agriculture and Life Sciences, Gödöllő, 2100, Hungary;

⁷ Department of Physiology, Veterinary Faculty, University of Santiago, Santiago, 71000, Biobío and the regions;

⁸ Corresponding author, e-mail address: kusza@agr.unideb.hu

Abstract. Understanding the responses of animals to seasonal heat stress on the genetic level has led to the identification of genes implicated in thermal stress reaction mechanisms. In this study, the relative gene expression of interleukin-10 (*IL-10*), an anti-inflammatory cytokine and biomarker for heat stress-mediated immune modulation, was observed during the summer and winter seasons in continental and tropical sheep breeds, namely, Hungarian Indigenous Tsigai and White Dorper, employing quantitative real-time polymerase chain reaction (qRT-PCR). Temperature-humidity indices (THI) were calculated to assess heat stress levels. The results indicate that *IL-10* CT-value was significantly higher during the summer, when heat stress prevails, in both sheep breeds compared to winter. While the White Dorper exhibited a higher numerical value for the stressor relative gene expression ratio (0.2) compared to the Hungarian Indigenous Tsigai (0.13), no significant differences in CT values were observed between breeds or among sexes. These findings suggest the immune-adaptive characteristics of the two sheep breeds during seasonal heat stress. The variation of *IL-10* gene expression levels between the two breeds can be attributed to their geographical origin: the White Dorper originating from arid subtropical South Africa and the Hungarian Indigenous Tsigai Sheep breeding in the seasonally harsh Continental climate, respectively.

Keywords: gene expression, heat stress, Hungarian Tsigai, *IL-10*, White Dorper

1 Introduction

As a major sector in the livestock industry, sheep production is key to ensuring global food security for the rapidly growing population. Its importance to low-income families all around the world as a source of food and livelihood cannot be overlooked as well [1]. With climate change projections pointing to worse scenarios of increased global temperatures in the years to come, it is essential to tailor-fit the livestock industry to a warmer and harsher environment by selecting animals with tolerance and adaptation traits [2]. Equipped with a robust knowledge of genes related to heat tolerance and adaptation, the livestock industry can keep up with the climatological challenges and sustain productivity.

Heat stress presents direct challenges to the well-being of animals that lead to an array of detrimental effects on their physiology, behavior, overall production performance, reproduction, and health [3]. In response to heat stress, the animal attempts to maintain homeostasis and re-establish homeothermy by invoking compensatory and adaptive mechanisms through behavioral and physiological changes to promote welfare and ensure survival [4].

The immune system of animals, in general, is one of the well-studied aspects of the physiological responses of livestock to heat stress. The multifaceted interplay of hormones and by immune system cells and factors during heat stress leads to complex, immune responses that involve rapid inflammatory reaction, autophagocytosis, immunosuppression, and shifting of immune equilibria,

among others [5-6]. Understanding and knowing these mechanisms and the factors involved under the victimization of reliable biological markers of heat response in animals with respect to immunity.

Cytokines are some of the most prominent actors in the immune system and, in this regard, the immune response to heat stress. Of them, the interleukin 10 (*IL-10*) plays a major role in modulating the other cytokines and effectors of the immune system that attempt to preserve its integrity and function while facing heat stress [7-8]. A deeper investigation of genes associated with heat tolerance and adaptation in livestock has raised the attention of researchers in recent years because of the increasing availability of genomic tools and capabilities. Reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR) is a reliable and key enabling technology for gene expression analysis, its rapid, cost-effective, and robust approach in gene expression analysis has encouraged researchers to use it for many purposes, including the detection and measurement of the relative expression of genes associated with heat tolerance and adaptation [9].

The influence of seasons on the expression of several heat stress-related genes in livestock was investigated in dairy cattle [10-11], sheep [12], and pigs [13]. This study aims to bridge the scarcity of comparable studies in sheep that intend to investigate seasonal effects on the expression of heat tolerance genes such as *IL-10* as well as a comparison of tropical and temperate breeds on the same subject.

In the present study, the relative gene expression of *IL-10* was determined in the Hungarian Indigenous Tsigai

sheep and White Dorper sheep in Hungary during the summer and winter seasons. The Tsigai sheep is an indigenous temperate triple-purpose breed in the Central-Eastern European region that has been bred and maintained as part of the Hungarian sheep sector for more than two centuries [14-15] while the White Dorper is regarded as a breed of Tropical origin that was developed in arid subtropical South Africa for meat purposes in the 1940s. The breed has just been introduced in Hungary in 2008 [16]. This study aimed at understanding the dynamics of the relative gene expression of *IL-10* during the two seasons, in the two breeds, and among the sexes, with particular emphasis on heat stress conditions and the further goal of expounding the adaptability of the two breeds to heat stress situations.

2 Materials and Methods

2.1. Sample Collection and Location

Four Hungarian Indigenous Tsigai sheep and 4 White Dorper sheep were utilized in this study as experimental animals. Of them, 2 are ewes, and 2 are rams for each breed. The animals were housed in conventional deep litter type housing with several pen separation devices and implement that allow adequate airflow and access to the immediate environment during the hot season and enclosure during the wet and cold seasons. Under the regular farm feeding regimen, the animals were provided clean drinking water. All animals that were part of the study were of similar age (2 to 3 years old), body weight (ewes: 45 to 55 kg; rams: 65 to 75 kg), and in optimal health, exhibiting no physical or anatomical abnormalities.

About 5 ml whole blood sample was obtained from the jugular vein of the same animals in RNAspreeq Animal Blood Tubes (QIAGEN, Hilden, Germany) and preserved at -70°C until further analysis.

The animals used in this study were kept in the Kónyas Experimental Station of Animal Husbandry of the University of Debrecen, located at 4002 Debrecen, Kónyas Terve 4, Hungary. The elevation is 127 m above sea level and site coordinates are 47.58° N and 21.38° E.

2.2. Climatological Data

Data on the climatic condition during the sampling day, which include temperature and relative humidity, were recorded from 12:00 to 13:00 in the afternoon and obtained from the Hungarian Meteorological Service station in the vicinity. To calculate the THI for each sampling day, the equation by Mader et al. [17] was used.

$$THI = (0.8 \times T_{db}) + \left(\frac{RH}{100} \right) \times (T_{db} - 14.4) + 46.1$$

T_{db} : Dry bulb temperature (°C), RH: Relative humidity (%)

The severity of heat stress in livestock is typically rated using the THI with ranges categorized as follows: no

stress (≤ 67), mild (68-74), moderate (75-78), severe (79-83), and extreme (≥ 84) [18]. Additionally, in order to provide a broader and more objective overview of the climatic cycle and changes in meteorological variables as the seasons change in the area and to give background reference to the climatological data on sampling days, the 30-month climatological records (August 2018 to January 2021) [19] measured by the adjacent government meteorological station, were obtained from the Hungarian Meteorological Service.

2.3. Quantification of relative gene expression levels using RT-qPCR

Following the manufacturer's instructions, total RNA was extracted from sheep blood using the RNeasy Protect Animal Blood Kit (QIAGEN, Hilden, Germany). Using a NanoDrop ND-1000 Spectrophotometer, RNA quality and quantity were assessed (Thermo Fisher Scientific, Waltham, MA, USA). Total RNA of about 100 ng was reverse transcribed into cDNA with specific primers (Table 1) by the qPCRBO cDNA Synthesis Kit (PCR Biosystems, London, United Kingdom). Forward and reverse primers (Table 1) were created with Primer Express v3.0.1 software (Applied Biosystems, Foster City, CA, USA) and confirmed for target identity with Primer Blast from the National Center for Biotechnology Information (NCBI) (Ye et al., 2012). A 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) was used to conduct quantitative real-time PCR (qPCR). The 10 μl reaction consisted of 5 μl PowerUp™ SYBR® Green Master Mix (Applied Biosystems, Foster City, CA, USA), 0.6 μl each of 10 μM forward and reverse primers, 1.3 μl dH2O (MillesiporeSigma, Burlington, MA, USA), and 2.5 μl cDNA (2 ng/μl). For relative expression studies, one housekeeping gene (glyceraldehyde 3-phosphate dehydrogenase (GAPDH)) was amplified alongside the target genes (*IL-10*). Quantitative PCR was done in triplicate for each sample.

Table 1. Detail of primers for qPCR analysis of the target genes

Target gene	Seq. Rank score	Primer	Prq. used (no. Op)
<i>IL-10</i>	NC_030945	F: TGATGCTTACAGGCTGAAAT R: CAGAAAACATGACACCTTCT	100
<i>GAPDH</i>	NC_010425	F: CTGGCAGGCGTATCCAT R: ACAGTCCTTCTGGGTTGAAAT	86

2.4. Data and Statistical Analysis

The relative expression of the *IL-10* gene was determined by calculating the values using the equation proposed by Pfaffl (2001). The method compared the expression of *IL-10* gene with the reference gene GAPDH, with their amplification efficiencies both

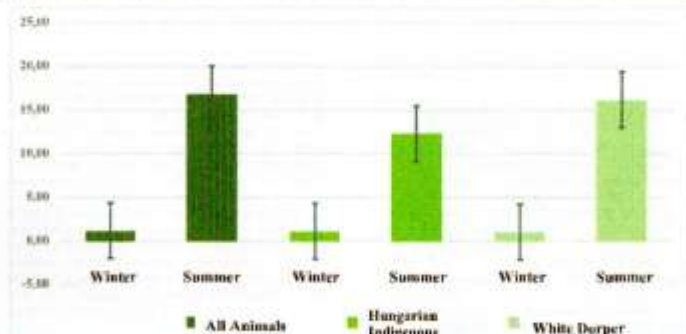


Figure 3. Bar graph of relative gene expression of *IL-10* in each season of the year with the winter season as the calibrator. The Y-axis is the relative gene expression in the two seasons. The X-axis is gene expression for all animals, and the two breeds studied. Different colors represent different breeds.

4 Discussion

Maintaining thermo-neutral conditions is one of the important requirements in ensuring the welfare and optimal production and reproduction performance of animals [26].

THI is considered as one of the most reliable measures and sensitive indicators of heat stress in animals, which integrates temperature and relative humidity parameters [27-28]. In this study, during the summer sample collection, THI was calculated to be high at 79.0, which indicates a state of severe heat stress. High temperature and low humidity are typical of summer seasons in the region. The average annual temperature in the immediate area was 11.66 °C for the period of 2011 to 2020 [29]. The average temperature recorded on the day of collection was 26.70 °C, a 136% deviation from the annual average. Factoring the daily maximum temperature and the relative humidity during the summer months of June to August [29], THI ranges from 69.67 to 88.90 for sheep, which indicates mild to extreme heat stress.

Consistent with the climatological data gathered and determined in this study, elevated THI in sheep during the summer seasons and hot periods that established a heat stress condition was also reported in Small tail Han sheep [30], Indian sheep [31], and Omani and Australian Merino sheep [32] where the animals exhibited increased respiratory rate, pulse rate, and rectal temperatures. Such signs are distinctive physiological indicators of heat stress response in animals [33-34].

High-THI conditions also influence metabolic and hematological parameters in sheep. Cortisol and biochemical parameters creatinine, zinc, and phosphorus were elevated during heat stress conditions and are highly correlated to THI levels in Suffolk sheep [35]. Sheep blood parameters chloride, sodium, phosphorus, total protein, tetraiodothyronine,

cholesterol, triglyceride, creatinine, cortisol, and glucose were likewise reported to be significantly increased by heat stress conditions in the study of Li et al. [36]. In the same way, values for red blood cell (RBC), hemoglobin (Hb%), and packed cell volume (PCV%) were significantly elevated in indigenous sheep subjected to heat stress [36].

The negative effects of heat stress on general welfare, comfort, and health could result in diminished immune function and increased incidence of diseases [37-38].

Primarily, the response of the immune system to chronic stresses, such as heat stress, begins with the stimulation of primary and secondary lymphoid and myeloid organs that catalyze the synthesis and release of immune cells and agents [5]. Studies show that in acute heat stress (brief and intense), immune activation happens, while chronic heat stress causes immune suppression [39]. Elevated cortisol levels, as a result of the activation of the hypothalamic-pituitary axis (HPA), alter various immune function elements that may increase the animal's vulnerability to diseases [40]. This effect is demonstrated in the induction of inflammation in acute heat stress and the suppression of release and synthesis of inflammatory cytokines and the consequent changes to the dynamics of balance on the levels of other cytokines, immune cells, and substances in chronic situation [41-42].

Cytokines are key mediators of the immune system's response to stressors and pathogens. Proinflammatory cytokines work to activate the innate immunity for the body's early response against infectious agents and support the activities of the cell-mediated immune response of the adaptive immunity to synergistically combat the pathogen of concern. However, specific cytokines modulate the previous cytokines to suppress the immune response and shift the modality of the immune system into adaptive immunity. The most powerful and significant immunomodulatory cytokine of this effect is *IL-10* [43].

In the present study, overall *IL-10* gene expression in Hungarian Indigenous Tsigan and White Dorper sheep were shown to be significantly higher ($P=0.015$) in animals under severe heat stress (summer) compared to those that experienced no heat stress condition (winter). This result is in accordance with the findings of Caroprez et al. [44], who demonstrated high *IL-10* expression in sheep experiencing hyperthermia. Similarly, in cattle, *IL-10* levels were increased in heat-stressed Holstein cows [10, 45], Italian Friesian cows [46], and Sahiwal cows [11].

The expression of *IL-10* in heat stress situations has various physiological and immunological indications that help the animal adapt and restore equilibrium. While it suppresses the proinflammatory response, it leads the immune regime from TH1 to TH2 that activates the adaptive immunity [47-48]. Sustained *IL-10* levels during heat stress and the extended period after the heat stress situation and strenuous activities have effectively controlled immune response to stress [49]. Ripley [50] has reported that *IL-10* activates heat shock protein 90 (HSP90). Heat shock proteins are molecular chaperones that are another important factor for protecting cells from damage, such as in heat stress situations and in antigen presentation to improve both innate and adaptive immunity [51]. *IL-10* expression by the experimental animals in this study suggests that there could also be accompanying HSP expression, which also aids in the immune response and endogenous protection against heat stress.

Furthermore, *IL-10*, in line with its anti-inflammatory action, has also been associated with the inhibition of cyclooxygenase (COX) and, consequently, prostaglandin E2 (PGE2), the principal downstream mediators of fever that are induced by inflammatory cells [52]. This would mean that the expression of *IL-10* during heat-stress situations is thermo-modulatory and helps reduce heat load as it eliminates endogenous heat production by fever pathways.

Though all the sheep in this study show significantly higher expression of the *IL-10* gene during the summer season, the inter-breed comparison reveals a numerically higher gene expression ratio among the White Dorper (16.2) against the Hungarian Indigenous Tsigan (12.70). Statistical analysis of the CT-values, however, yielded no significant difference between the two breeds on the same subject. The two breeds, despite having different geographical origins, manifest comparable thermo-tolerance evident in the *IL-10* mRNA expression that bears no significant difference.

The Hungarian Indigenous Tsigan Sheep, being a temperate indigenous breed that thrived in the continental climate for centuries [15, 53], elucidated heat stress response in the distinctively harsh and hot summer environment of the region.

In a similar manner, the White Dorper sheep that is considered tropical in origin, maintained its heat stress responsive characteristic as it experienced the hot summer season in the study location when it was introduced a few years ago. Despite being present in Hungary for not a long time, the breed's tropical characteristics made acclimatization manageable for it to thrive during the hot summer months in the area.

Even though *IL-10* is a prominent immunological biomarker in heat stress-related studies in domestic animals, comparative studies of its mRNA expression across breeds, particularly between tropical and temperate breeds, are very scarce, especially on sheep.

In contrast to the findings of the present study, various related studies reported differences in *IL-10* gene expression among different breeds in domestic animals, such as cattle, during heat stress. Previous studies showed significantly higher *IL-10* gene expression in Sahiwal cows [11] and Karan Fries cows [54] compared to other breeds that include temperate ones. Citing other related thermotolerance immune biomarkers such as heat shock proteins (HSPs), Sahiwal, a tropical cattle breed, also exhibited higher mRNA expression of HSP90 [55] and HSP70 [56] compared to the temperate and tropical cross, Frieswal. Following this trend, the Caribbean sheep breed Pellibury was found to be less susceptible to heat stress and with increased HSP70 concentration compared to Sahiwal [57]. A comparative study in pigs revealed better cell viability in the tropical Croatian pigs than the temperate Large White pigs, with a significant increase in the expression of HSPs. Interestingly, however, there was no difference in mRNA expression of HSP70.2 and HSP90 [58] in agreement with the findings of the current study.

The increase in *IL-10* concentration can result in the upregulation of HSPs gene expression in peripheral blood mononuclear cells, consequently leading to increased HSP concentration in circulation [50].

The equivalent heat tolerance in the Hungarian Indigenous Tsigan to the tropical breed White Dorper indicated by its *IL-10* gene expression in this study can be attributed to its indigeneity in the area that is shown in its hardiness and suitability to local environment. Its exposure to the local agro-climatic conditions across generations through centuries helped it adapt to the harsh summer months in the region. Its success in variable climatic conditions is proven by its wide distribution in the Central-Eastern European and Balkan regions being integrally used in sheep breeding programs confirmed by relatively low to moderate levels genetic variation among country populations [33, 59]. The tenacity of indigenous breeds in extreme climatic conditions can be ascribed to their physiological and genetic adaptations that have arisen over generations of slow modification and adaptation to environmental challenges [60, 28].

On another note, the excellent thermotolerance character of the White Dorper sheep, according to the parameters of this study, suggests its suitability for the harsh summer heat stress conditions in the area, despite its relatively short period of habitation. Tropical breeds can thrive comfortably in temperatures as high as 38 °C and produce adequately by virtue of thermoregulatory processes [61]. This characteristic was exhibited by Molpura cows from the Indian semi-arid regions [62] and Santa Ines and Morada Nova sheep in Brazil [63]. Performance-wise, Dorper raised in the same area under Hungarian rearing conditions proved to have a good production and reproduction performance as reported in a study by Buda et al. [16].

In a pilot study evaluating reproductive performance and health indicators in the same area in Hungary, genotype differences were found in incidences of clinical mastitis (higher in Friesian), lameness (higher in Friesian), and litter size (lower in White Dörper), while no differences were observed in conception and lamb survival rates [64]. It provides an overview of the variability of immune status among the breeds of interest, which merits the conduct of further investigation as to how heat stress could affect these incidences and the role of *IL-10* in such health challenges and reproductive performance. Finally, in contrast with the findings of Li et al. [10], that reported better high-temperature tolerance in ewes over rams, the present study finds no sexual differences. The absence of sexual differences in the mRNA expression of *IL-10* in this study implies that thermotolerance and heat stress immune responses in both breeds are not influenced by sexual factors.

5 Conclusion

Overall, the study has demonstrated higher relative gene expression of the *IL-10* gene during the summer season in both the Hungarian Indigenous Friesian and White Dörper when heat the animals experienced heat stress conditions in contrast with the winter season. Implicated as a molecular biomarker for heat stress response, the significant relative gene expression of *IL-10* indicates immune modulation and shift to adaptive immune response. There has been no significant difference in the *IL-10* gene expression between the two breeds. While sexual factors do not affect *IL-10* gene expression, its expression can potentially enhance physiological adaptive mechanisms to avoid cellular damage during heat stress conditions in both breeds, regardless of sex.

Owing to their origins, the comparable heat stress response through the expression of the *IL-10* gene proves that both breeds, continental and tropical in origin, are adaptive to the region's environmental conditions and could be utilized in breeding programs to improve the immunity, resiliency, and production performance of sheep, even in variable climatic conditions.

Further study on the links of genetic adaptability, physiological reactions, immune response, breeding programs, animal health, and productivity must be conducted to unravel complex mechanisms of homeostasis and animal performance in the face of heat stress, particularly involving *IL-10* and related biomarkers.

Acknowledgement: The research was funded by the bilateral S&T cooperation programme, within the project "Effect of heat stress on physiological types of sheep using DNA and MS-E based methods" from the National Development, Research and Innovation Fund (2011-1-2.4-1/2011-2014) also by the EFOP-16.2-16-2017-40001 project, entitled, "Complex rural economic development and sustainability research and development of the service network at the Csepelkősző Farm". R.P. and P.K.A. were supported by Tempus Public Foundation

within the Hungarian Munkajuttatás Program, Authors would thank Csaba Rácz for providing climatic data during the sampling period, János Oláh for helping in sampling and Andrić Števo for his valuable comments in preparation of manuscript.

References

1. H. Benthahy, A. Ahmed, X. He, L. Asog, & Y. Ma. *Animals*, **9**(3), 75 (2019).
2. I. Mirtal. *J. Anim. Sci.*, **95**, 1780-1787 (2012).
3. V. P. Rashamol, V. Sejian, M. Bagath, G. Krishnan, P. R. Archana, & R. Bhatta. *J. Anim. Behav. & Biomet.* **6**(3), 62-71 (2018).
4. S. Indu, V. Sejian, D. Kumar, A. Poreck, & S. M. K. Nagvi. *Trop. Ani. H. & Prod.*, **47**(8), 1487-1495 (2015).
5. R. J. Collier, J. L. Collier, S. P. Rhoads, & L. H. Baumgard. *J. Dairy Sci.*, **91**(3), 445-454 (2008).
6. M. Bagath, G. Krishnan, C. Devaraj, V. P. Rashamol, P. Pragna, A. M. Lees, & V. Sejian. *Anim. Res. Vet. Sci.* **126**, 94-102 (2019).
7. R. Sabot, G. Griza, K. Wyszczotko, S. Kowal, F. Witte, K. Walk, & J. Gęgotek. *Cyt. & Onco. Rev.*, **21**(5), 331-344 (2016).
8. K. N. Cooper, D. G. Blizard, & E. M. Riley. *J. of Inmua.*, **18**(9), 5771-5777 (2008).
9. H. D. Van Gelder, K. E. Vrana, & W. M. Freeman. *Ann. Entomol. Soc. Am.*, **44**(5), 619-626 (2008).
10. F. J. Zhang, X. G. Wang, J. F. Wang, D. Zhou, W. Zhang, C. Zhao, Y. X. Jiao & Y. H. Zhai. *J. Anim. Sci.*, **92**(7), 3026-3034 (2014).
11. S. Grewal, A. Aggarwal, & M. N. Adhosen. *Bio. Rhyth. Res.*, **52**(8), 1229-1239 (2019).
12. Z. Li, M. Chu, Q. Li, M. Jin, X. Pei, L. Ma, L. Zhang, & C. Wei. *Animals*, **9**(6), 387 (2019).
13. X. H. Ju, H. J. Xu, Y. H. Yong, F. L. An, P. R. Jiao, & M. Lian. *Animals*, **8**(9), 1462-1468 (2014).
14. C. Marley. Evaluation of the growing performance of rare breed sheep. *Szent István University* (2014).
15. S. Kasza, I. Nagy, T. Nemeth, A. Molnár, A. Javor, & S. Kakovics. *Arch. Anim. Breeding*, **53**(3), pp.309-317 (2010).
16. C. Bostai, D. Gasvóldi, A. Kovacs, F. Negrut, J. Oláh, L. T. Czirner, S. Kusos, & A. Javor. *Anim. Sci. & Biotech.*, **46** (1) 346-349 (2013).
17. T. L. Mader, M. S. Davis, & T. Brown-Brandl. *J. Anim. Sci.*, **84**(3), 712-719 (2006).
18. B. B. Lewis Baldo, A. M. Swinburne, J. Benwick, S. T. Lau, & W. H. E. J. van Weeren. *Animal Biotechnology*, **9**(1), 4 (2017).
19. L. Schüller, O. Barónad, & W. Hentschew. *Theriogenology*, **82**(8), 1050-1057 (2014).
20. J. Ye, G. Contreas, J. Zureckaya, I. Cukuttsche, S. Ruten, & T. L. Madden. *BMC. Biostatistics*, **13**, 134 (2012).
21. M. W. Pfaffl. *Nucleic Acids Res.*, **29**, e45 (2001).
22. P. Kic. *Animals*, **12**(15), 1895 (2022).
23. I. Schüller, O. Barónad, & W. Hentschew. *J. Dairy Sci.*, **96**(12), 7731-7738 (2013).

24. M. Akhtar, S. Guo, Y. Qiao, A. Zahner, A. Shaikat, Y. Chen, T. Umar, P. G. Deng, & M. Guo. *Acta Tropica*, **207**, 105458 (2020).
25. R. Development Core Team. *R: A Language and Environment for Statistical Computing*, R Foundation for Statistical Computing, Vienna (2009).
26. J. W. West. *Journal of Dairy Science*, **86**(6), 2131-2144 (2003).
27. S. D. Rathwa, A. A. Vastava, M. M. Pathan, S. P. Madhwa, V. O. Patel, & A. M. Pande. *Veterinary World*, **10**(6), 650-654 (2017).
28. V. Sejian, R. Bhatta, J. B. Gaughan, F. R. Dunshea, & N. Lavrenko. *Animal*, **12**(e2), e411-e414 (2018).
29. Hungarian Meteorological Service. *Meteorológiai Szolgálat*. <https://odp.met.hu/climate/observations/hungary/index.html> (2022).
30. P. K. Li, Y. Yang, K. Jena, C. H. Xia, S. J. Lv, & W. B. Wu. *Trop. Anim. Health & Prod.*, **50**(8), 1892-1901 (2018).
31. K. M. Singh, S. Singh, J. Ganguly, A. Ganguly, R. K. Nachapatti, A. Chatter, & H. K. Narula. *Soc. Ram. Res.*, **141**, 113-117 (2016).
32. A. Siklándi-Kovács, E. H. Johnson, & G. Mahgoub. *Soc. Ram. Res.*, **49**(2), 193-198 (2003).
33. J. Blackshaw, J. & A. Blackshaw. *Am. J. of Exp. Agric.*, **34**(2), 285 (1994).
34. S. Inlu, & A. Poreck. *Int. J. of Pract. Trends in Sci. & Tech.*, (2015).
35. J. Auzanaitė, I. Polkienė, V. Ausanaitienė, R. Antanaitis, W. Boumaguaz, & R. Žilinskis. *Indian J. of Anim. Res.* (2021).
36. M. S. Rana, M. A. Hashem, M. N. Sakib, & A. Kumar. *African Science*, **12** (1): 91-99 (2014).
37. S. L. Cartwright, M. McKechnie, J. Schaefer, A. M. Livrenois, & B. A. Mallard. *BMC Veterinary Research*, **17**(1) (2021).
38. U. Bernabucci, N. Lavrenko, L. R. Baumgard, R. P. Rhoads, B. Rotchi, & A. Nardone. *Animal*, **4**(7), 1167-1183 (2010).
39. A. M. Livrenois, B. A. Mallard, S. L. Cartwright, & A. Clainos. *Scientific Reports*, **11**(1) (2021).
40. N. Lavrenko, U. Bernabucci, D. Scallia, L. Bonaiuto, P. Mures, & A. Nardone. *J. of Dairy Sci.*, **89**(12), 4600-4612 (2006).
41. L. Gu, Y. Wang, H. Wang, J. Dong, Z. Li, J. Li, C. Qian & J. Li. *BMC Veterinary Research*, **16**(1) (2020).
42. M. Bagath, G. Krishnan, C. Devaraj, V. P. Rashamol, P. Pragna, A. M. Lees, & V. Sejian. *Anim. Res. in Vet. Sci.*, **126**, 94-102 (2019).
43. S. Jabra, V. Sejian, M. Bagath & R. Bhatta. *Pertanika Journal of Tropical Agricultural Science*, **39**(4) (2016).
44. M. Caroprete, M. G. Tibbeni, G. Amicciandino, M. Albetto, A. Musico & A. Sevi. *J. of Dairy Sci.*, **97**(1), 4347-4358 (2014).

45. I. M. T. Thompson, S. Tao, A. P. A. Montello, K. C. Ayng, & G. F. Dahl. *J. of Dairy Sci.*, **97**(12), 7426-7436 (2014).
46. M. Caroprete, A. Marzano, G. Estroren, S. Walfordem, M. Albetto & A. Sevi. *J. of Dairy Sci.*, **92**(6), 2796-2803 (2009).
47. E. Cakragli & I. Elenkov. *Annals of the New York Acad. Sci.*, **1049**(1), 62-76 (2006).
48. S. H. Sulhagui, D. Koca, J. Park, M. Khan & K. Shim. *Scientific Reports*, **10**(1), 18872 (2020).
49. F. A. Bingle, C. C. Oryemwote, J. J. Ewagha, C. O. Akosile & D. E. Agboluaje. *Int. J. of Bio. & Chem. Sci.*, **11**(3), 947 (2017).
50. R. J. Rigley, A. Stephenson, D. A. Isenberg & D. S. Jadhav. *Anim. Immunology*, **97**(2), 226-231 (1999).
51. P. B. Anchari, J. Akera, F. Pragna, M. K. Vidy, P. A. Akshai Niyas, M. Bagath, G. Krishnan, A. Maitteenan, V. Breen, E. K. Kumar, V. Sejian & R. Bhatta. *J. of Dairy, Vet. & Anim. Res.*, **5**(1) (2017).
52. H. Niwa, T. Otsuka, K. Izuhara, K. Yamaoka, K. Ohtsuka, T. Tanabe, S. Hara, Y. Nemoto, Y. Tanaka, H. Nakashima & Y. Niho. *Blood*, **89**(5), 1197 (1997).
53. S. Kusra, E. Gyarmathy, J. Dubravski, I. Nagy, A. Javor, & S. Kakovics. *Czech J. Anim. Sci.*, **54** (10), 468-474 (2010).
54. A. A. Sheikh, A. Aggarwal, & O. Awif. *J. of Thermal Bio.*, **56**, 68-76 (2016).
55. R. Dub, B. Sajjan, U. Singh, S. Kumar, R. Singh, G. Seegar, & A. Sharma. *Genes*, **53**(2), 435-440 (2014).
56. V. Bhauprakash, U. Singh, G. Seegar, B. Sajjan, B. Bhatia, T. V. Raju & R. Deb. *J. of Thermal Bio.*, **59**, 18-25 (2016).
57. R. D. Ramona, A. Munteanu Parola, H. H. Mornaldu, A. D. Rodriguez & J. Hernandez. *Cerin Trop. Anim. Health & Prod.*, **45**, 1691-1696 (2013).
58. J. C. Bamhou, J. I. Gourline, R. Gredin, N. Vachery & D. Renaudeau. *Trop. Anim. Health & Prod.*, **43**(8), 1535-1541 (2011).
59. S. Kasza, A. Jankovics, J. Ramjak, I. Nagy, A. Javor, & S. Kakovics. *Small Rum. Res.* **99** (2), 130-134 (2011).
60. M. M. Rojas-Dominguez, A. P. Najib-Basham, I. Harizan, & S. A. Wozniak. *Climate Risk Management*, **16**, 145-163 (2017).
61. R. Gandhi & S. Arjya. *Indian Dairy Anim.*, **68**, 182-187 (2016).
62. V. Sejian, V. P. Maurya, & S. M. Nagvi. *Trop. Anim. Health Prod.*, **42**, 1763-1770 (2010).
63. T. P. Das, E. Silva, J. N. Costa Turcato, C. A. Toraldo Marques, M. J. de Araujo, I. R. Bezerra, D. Kumar Dhanasekaran, & V. Sejian. *J. Therm. Biol.*, **59**, 39-46 (2016).
64. D. Gawaian, C. Rudri, L. T. Cristler, N. Cstanzu, A. Javor & S. Kusra. *Anim. Immunology J. of Anim. Sci.*, **28**(4), 599 (2015).

CALABARZON, and Ilocos) in terms of live weight production; 2) to conduct economic cost-benefit analyses of the ISPRFE project, which was given to the industry stakeholders in the three regions for 2022–2023, and 3) to estimate the societal national investment requirements from 2025 to 2028 to restore pig production to pre-ASF 2018 levels. The choice of study was designed to ascertain the impact of the pig repopulation program on the top two locally hit regions and one slightly hit region to determine pig repopulation impacts and to guide future pig repopulation policies. Before the ASF hit in 2018, Central Luzon and CALABARZON were the top two pig-producing regions. Ilocos Region is a major pig-producing region in the north. The findings of this study could contribute to policymaking, industry resilience, and long-term ASF mitigation strategies.

2. Materials and methodology

2.1. Model specification and justification

The SARIMA model, an extension of the classical ARIMA approach, is designed to model time series data that exhibit both trend and seasonal patterns. While the standard ARIMA model captures autoregressive and non-stationarity through autoregression (AR), differencing (I), and moving average (MA) components, adds a seasonal layer, making it well-suited for data with periodic fluctuations.

Formally, a model is denoted as ARIMA (p, d, q) (P, D, Q) (s), where:

- (p, d, q) represent the non-seasonal AR, differencing, and MA terms;
- (P, D, Q) represent the seasonal AR, differencing, and MA terms;
- s denotes the length of the seasonal period (e.g., 4 for quarterly data, 12 for monthly data).

According to [Jin et al. \(2015\)](#) and [Ponrajapornwittaya et al. \(2023\)](#), the model extends the basic ARIMA formulation by incorporating seasonal autoregressive and moving average operators, which allows the model to better capture recurring seasonal behaviors observed in the data. This model was selected due to its ability to handle both non-stationarity and seasonal structures in time series data. This is particularly relevant for pig production data, which often shows strong seasonal patterns influenced by biological cycles, market demand, and environmental conditions. The model's strength lies in its ability to utilize historical values and errors—including those from previous seasons—to generate reliable forecasts. The adoption is also consistent with previous applications in livestock forecasting and epidemiological modeling (e.g., [Rastanjanjan & Mariani, 2022](#); [Yinso & Akhtar, 2021](#); [Ivanovic-Lucan et al., 2023](#); [Ponrajapornwittaya et al., 2023](#)), where seasonal effects were found to significantly affect production and disease dynamics. Its flexibility and interpretability make it a powerful tool for understanding and predicting fluctuations in regional pig production, especially in the context of disruptions such as ASF.

2.2. Data description and study scenarios

This study employs quarterly regional pig production data from the Philippine Statistics Authority (PSA), covering the period from the first quarter of 2008 (2008Q1) to the first quarter of 2024 (2024Q1). As noted by [Jin et al. \(2015\)](#), a minimum of 50 observations is recommended for reliable time series modeling, with over two decades of quarterly data, amounting to more than 90 observations. This study meets the necessary threshold for robust SARIMA forecasting.

To evaluate the impact of national shocks and policy interventions, two distinct analytical scenarios were developed:

1. No ASF Scenario—Includes data from 2008Q1 to 2018Q3, representing a hypothetical baseline in which ASF had not yet emerged.

2. No-ISPRFE Scenario—Includes data from 2008Q1 to 2021Q4, capturing the condition prior to the launch of the ISPRFE program.

Before modeling, the dataset was preprocessed to detect anomalies, ensuring the accuracy and consistency of the time series. Regions were categorized as either ASF-affected or non-affected to facilitate comparative forecasting and policy evaluation between impacted and unimpacted groups. Supplementary data were also obtained from ISPRFE program implementation reports, providing essential context for interpreting government interventions and shaping the subsequent cost-benefit analysis.

2.3. Model estimation

Given the quarterly nature of the dataset (i.e., seasonal period $s = 4$), model estimation was conducted using the `autoarima` function ([Hyndman & Athanasopoulos, 2020](#)) from the `forecast` package in R ([Core Team, 2021](#)) and `AutoARIMA` ([Hyndman, 2020](#)). This function automates the selection of optimal model parameters by searching across a wide grid of candidate models and identifying the best-fitting structure based on information criteria—specifically, the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC). This approach ensures that the final model is both parsimonious and statistically robust.

Model diagnostics were conducted to validate adequacy. Residuals were examined for autocorrelation using autocorrelation function (ACF) and partial autocorrelation function (PACF) plots, and the Box-Cox test was employed to confirm that the residuals approximated white noise. These procedures ensured that the selected models captured the underlying data structure effectively and were well-suited for generating reliable forecasts across different policy and outbreak scenarios.

2.4. Economic evaluation: cost-benefit analysis (CBA)

To evaluate the economic viability of the ISPRFE program, a Cost-Benefit Analysis (CBA) framework was employed. This approach compares the present value of projected benefits from pig repopulation against the present value of implementation costs.

$$\text{Cost-Benefit Analysis Ratio} = \frac{\text{Present Value of Benefit}}{\text{Present Value of Cost}} \quad (1)$$

A Net Present Value (NPV) calculation was also performed:

$$\text{NPV} = \sum_{t=0}^T \frac{WCF_t}{(1+r)^t} \quad (2)$$

where NPV = net present value, WCF_t = net cash flow generated by the proposed project in year t , and r = discount rate. These indicators help determine the financial justification for continued or expanded public investment in pig repopulation efforts.

The concept of CBA is rooted in early work by [Dupont \(1962\)](#), with further development in the field of agricultural economics ([Whee, 1975](#)). In this study, CBA was informed by actual program data and pig production forecasts derived from ARIMA modeling, thus integrating empirical forecasting with economic assessment.

3. Results and discussion

Two sets of results were generated and compared with those of PSA production. First, the ARIMA forecasting results for a scenario with no ASF using datasets from 2008Q1–2018Q3 (75 data points). Central Luzon, CALABARZON, and Ilocos Region. Second, a dataset from 2008Q1 to 2021Q4 (98 data points) was used to forecast the pig production scenario, representing the period before the implementation of the ISPRFE project. [Fig. 1](#) shows the map of the three regions in the Philippines.



Fig. 1 Central Luzon, CALABARZON, and Ilocos Region. Source: RA Information and Communications Technology Service (ICTS)

3.1. Results of the Augmented Dickey-Fuller (ADF) test for stationarity under No ASF and ASF scenarios

To assess the appropriateness of SARIMA modeling, all the time series data set were subjected to the ADF test to assess stationarity. Pre-steps of the series, the null hypothesis of non-stationarity was not rejected at level, but was rejected after first differencing, indicating that the series became stationary (upon differencing, ADF Scenario data were stationary after the second differencing (not shown). A summary of these results is presented in [Table 1](#).

3.2. Model estimation, diagnostic checking and forecasting

3.2.1. Central Luzon

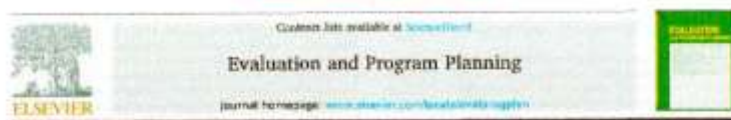
For the Central Luzon an ASF scenario, the model chosen was SARIMA (1,1,1) (0,1,0), with a MAPE of 4.13 and an AIC of 1464.18 ([Table 2](#)). The ACF plot in [Fig. 2](#) shows that only one lag exceeds the range, which indicates a feasible model. As shown in [Table 3](#), the Box-

Table 1 The Augmented Dickey-Fuller (ADF) Test for stationarity

Variable	No ASF scenario		ASF scenario	
	A ₁ (t-1)	ADF (t) difference	A ₁ (t-1)	ADF (t) difference
Central Luzon	-1.1996	-4.0222**	-0.20137	-5.4320**
CALABARZON	-2.0122	-4.1202***	-1.1271	-2.2075
Ilocos Region	0.7515	-4.0240***	0.1668	-3.9757**

***, ** and * represents stationarity at 1, 5, and 10% levels of significance respectively. Note: Central Luzon, CALABARZON, and Ilocos Region No ASF denoted non-stationarity at 1% after 2nd difference.

Qing test's p-value of 0.57 indicated white noise (or no autocorrelation). A low p-value (typically less than 0.05) indicates that the null hypothesis of no autocorrelation is rejected, which suggests the presence of autocorrelation. On the contrary, a high p-value means there is not enough evidence to reject the null hypothesis, indicating an insignificant



Ex-post impact analysis of the Philippine pig repopulation program

Lacy Nel B. Abay^a, Ruth M. Soroco^b, Miguel V. Golegno^c, Alvin Paul J. Dirain^d,
Roland Y. Fajardo^{a,e,f}, Joseph Frank Mgyoa^a

^a Department of Agriculture - National Livestock Program, Malabon Road, Diliman, Quezon City, 1500, Philippines
^b University of Southern Mindanao - College of Veterinary Medicine, Davao, Cotabato, Philippines
^c Department of Anatomy and Histology, University of Southern Mindanao, Davao, Cotabato, Philippines
^d Marine Biotechnology - School of Agriculture and Forestry, University of Southern Mindanao, Davao, Cotabato, Philippines
^e Department of Statistics, University of Southern Mindanao, Davao, Cotabato, Philippines
^f Institute of Statistics, University of Southern Mindanao, Davao, Cotabato, Philippines

ARTICLE INFO

Keywords:
 African swine fever
 Pig production
 SWARA
 Cost-benefit analysis
 Pig repopulation

ABSTRACT

The 5-year Integrated National Swine Production Initiative for Recovery and Expansion (INSPIRE) program is the national repopulation and recovery program of the Philippine Department of Agriculture-National Livestock Program, offering a mix of interventions aimed at reviving the swine industry, which was significantly impacted by African Swine Fever (ASF). This study focused on the interventions provided in the three regions with the highest allocations from fiscal years (FY) 2022 and 2023: Central Luzon, CALABARZON, and the Davao region, a combination of SWARA modeling and financial analysis was employed to ascertain the impact of the repopulation program.

Two scenarios were created and assumed a plausible without African swine fever outbreak, using datasets from 2000Q1 to 2019Q4, whereas the other reflects the situation prior to the implementation of the INSPIRE program, which utilizes datasets from 2000Q1 to 2020Q4.

The study revealed the impact of the INSPIRE program in three three regions, revealing that Central Luzon and CALABARZON significantly benefited from the program. Without the INSPIRE program, pig production in Central Luzon would have been edged out by the second quarter of 2022 (2022Q2), whereas CALABARZON would have faced a similar scenario by the first quarter of 2023 (2023Q1). In contrast, the INSPIRE program had a lesser impact in the Davao Region, as it was affected by ASF later.

The cost-benefit analysis and net present value of the program investment yielded favorable ratios that indicate their financial viability and success. Based on the impact analysis, it is recommended that the swine repopulation program be continued beyond 2025 with increased funding.

This is the first study in the Philippines which utilizes the SWARA framework to evaluate the impact of a pig repopulation program.

1. Introduction

African swine fever (ASF) is a highly contagious viral disease that affects domestic and wild pigs and causes significant illness and mortality. It is not harmful to humans or other animal species, but it can cause severe economic losses in affected areas due to the death of pigs, restrictions on movement of pigs and their products, and added costs of control measures.

1.1. Global, Asian, Philippine and Philippine regional perspective of ASF

[Ponlaipaikulchai et al. \(2021\)](#) and [Ponlaipaikulchai et al. \(2022\)](#) reported

on the current situation of ASF (globally, Asia and in the Philippines). Other studies have given global ASF, Philippine context, and regional context such as [Hsu et al. \(2022\)](#), [2022b](#), [2024](#) in CALABARZON, [Garcia et al. \(2024\)](#) in Central Luzon and [Ligaya et al. \(2022\)](#) in Davao Region. [Ponlaipaikulchai et al. \(2021\)](#) provided a detailed global overview of ASF globally and in the Philippines. On the Philippine situation, the study outlined current ASF surveillance, prevention, and control strategies in the Philippines. [Hsu et al. \(2022a\)](#) reported a clear seasonal pattern with higher frequencies of ASF outbreaks occurring from August to October and lower frequencies during April to May. It added that in terms of space, disease was more severe in the northern island of Luzon, particularly during the second half of the year. They

concluded that spatial and temporal patterns can inform disease prevention and control efforts in the future, and help prioritize program focus and optimize the impact of potential ASF outbreaks. In another follow-up study, the findings of [Hsu et al. \(2022a\)](#), [Ponlaipaikulchai et al. \(2021\)](#) suggested that well- or contained land, biosecurity biosecurity protocols, and movement of personnel were the top risk factors involving ASF spread among farms in general. For commercial farms, they added that commercial vehicles and personnel were also important, whereas for backyard farms, the introduction of new pigs, environmental contamination, and poor feeding quality were relevant risk factors. Garcia et al. (2022) gave sequenced 125 blood samples from Central Luzon, Philippines from January to April 2022. The results of their study revealed close phylogenetic relationships among ASF isolates from Ukraine, Myanmar, India and the rest of the Philippines. The results of the study revealed close phylogenetic relationships among ASF isolates from Vietnam, Malaysia, India and the Philippines.

In July 2019, the Philippines reported its first cases of ASF in final production near Marikina City ([DO, 2019](#)). The virus subsequently spread to other provinces, including Bulacan, Pampanga, Nueva Ecija, and Cavite. The ASF outbreak significantly impacted Philippine pork production, leading to the culling of more than 200,000 pigs. As a result, the growth rate of pork production dramatically decreased by 26.5% in 2021 ([Philippine Statistics Authority, 2022](#)). In addition to its devastating effects on pig farmers, ASF also leads to higher pork prices, severely affecting vulnerable consumers and livelihoods ([Cisneros et al., 2022](#)). In response to the ASF outbreaks, the Philippine government implemented various animal and public health strategies ([Cisneros et al., 2022](#)), such as the Individual Farming and Movement (IFM) plan for ASF, which designates different zones based on ASF risk levels and restricts movements between regions.

Libing from the blueprint of the National Food and Meat Security Evaluation Program in the Philippines ([Golegno et al., 2011](#)), a set of actions referred to as the 1-2-10 protocol was conceptualized and implemented to contain the spread of ASF. Recently, DA Administrative Order (AO) 6, Series of 2024 revised 1 kilometer in the 1-7-10 protocol of DA Administrative Order (AO) No. 22, Series of 2020-2020 was the essential component of the revised protocol include the rapid culling of domestic pigs within the 500-meter radius of ASF-infected farms, implementing active surveillance activities and testing within the 7 km radius, and requiring pig farms within the 10 km radius to submit a mandatory report on disease surveillance. The stamping-out of pigs within 500 m from the infected premises (the quarantine zone) is mandated to be completed within five days or less, regardless of the population.

On 10 May 2021, Presidential Proclamation No. 1143 signed by President Rodrigo R. Duterte declared a State of Calamity throughout the country for one year, acknowledging the agency of mitigating economic losses and ensuring food supply ([DO, 2021](#)). The proclamation allowed local government units (LGUs) to allocate funds for quick response activities and the central offices of the Philippines to provide support in maintaining swine and order in affected areas.

Additionally, the Ready ASF-to-Rescue Program (Ready ASF), a National African Swine Fever Prevention and Control Program, was launched in 2021. The program aimed to prevent and control ASF through surveillance, monitoring, and repopulation efforts. It involved information dissemination, biosecurity measures, and collaboration with local government units.

1.2. Integrated National Swine Production Initiative for Recovery and Expansion (INSPIRE) Program

To address the decline in pig production due to ASF, the INSPIRE program was conceptualized. The 5-year INSPIRE program is the national repopulation and recovery program of the Department of Agriculture-National Livestock Program (DA-NLP), which offers various interventions in jumpstart and revive the severely affected pig industry

following the guidelines of the DA Memorandum Circular No. 24 series of 2020 and to encourage investments and revivals. The ASF crisis, however, presents the opportunity to rebuild, modernize and restructure the industry for greater efficiency, sustainability and disease resilience. While the primary focus of the program is on increasing pig productivity and the broader base, the program also pays particular attention to cleaning and consolidation strategies, specifically for smallholder farmers, to facilitate technical support services and other interventions along the value chain. In support of the repopulation efforts, the program also includes the implementation of biosecurity plans as outlined in the Ready ASF Program.

Government financial institutions have been tapped to provide concessional and flexible loan terms to commercial pig farmers to increase recovery. According to the latest Philippine Statistical Authority (PSA) data, commercial pig raises have lost approximately 30% of their breeder animals.

Moreover, the program also focused on establishing an acceptable insurance system through the Philippine Crop Insurance Corporation to encourage and restore the confidence of pig farmers. For 2022, the INSPIRE program had a budget of PHP2.75 billion (\$46 million), whereas for 2023, the budget was increased to PHP2.95 billion (\$52 million) to implement its two major components: Coordinated Repopulation and Recovery of Breeder Sows.

1.3. Review of related literature

Most impact analyses in scientific literature discuss animal health program strategies. There is no mention of impact analyses of animal repopulation programs using the time series Seasonal Autoregressive Integrated Moving Average (SARIMA) modeling (which is the unique contribution of this paper to scientific community). This research paper aims to contribute to the literature in addressing this gap and by proposing a new method of estimating impact using existing tools (e.g., SARIMA modeling and cost-benefit analysis). For animal health programs, [Toussaint et al. \(2022\)](#) developed a stochastic model combining transmission between wild boar and domestic pigs, which was tailored to specific data corresponding to different phases describing epidemic progression. Their model was then used to produce forward projections describing the likely temporal evolution of the epidemic under various disease management scenarios of ASF.

[Garcia et al. \(2022\)](#) reported that a cost-benefit analysis based on significantly improved performance parameters demonstrated that Mycoplasma hyopneumonae vaccination was economically attractive. [Garcia et al. \(2020\)](#) conducted a cost-benefit assessment of Salsalidol control strategies in pigs raised in the District Kingdom. The study suggested that the cost of epidemiological surveillance, the savings for off-inventories, even if the effectiveness could be improved. In addition, to achieve a net cost benefit, reducing the cost of interventions is essential. [Garcia et al. \(2020\)](#) reported that vaccination with a highly efficacious vaccine may be a cost-effective strategy to control PRRS, especially when vaccination costs are not independently staggered and show a no future adverse economic impact, such as trade restrictions. [Golegno et al. \(2021\)](#) applied partial budget method and estimated the benefit of vaccinating new born piglets continuously to control porcine reproductive and respiratory syndrome virus control.

[Crisola et al. \(2017\)](#) conducted a quantitative assessment of the social and economic impacts of ASF outbreaks in northern Uganda. The study indicated that the causal relationship between the social and economic impacts of ASF outbreaks in smallholder systems is complex. Pigs are often kept as pasture investments to use when developing countries other than as active working capital, complicating economic analysis and challenging disease control arguments based on only standard economic models.

This paper examines the economic impact of pig repopulation. The purposes of this research paper are as follows: 1) to conduct an in-depth evaluation of the impact of INSPIRE in three regions (Central Luzon,

^{*} Corresponding to University of Southern Mindanao-College of Veterinary Medicine, Davao Region, Eastern 9407 Cotabato, Philippines.
 E-mail address: rlfajardo@usm.edu.ph (R.Y. Fajardo).

<https://doi.org/10.1016/j.eppp.2023.100975>

Received 8 September 2023; Received in revised form 10 July 2025; Accepted 7 August 2025

Available online 13 August 2025

0145-7189/© 2025 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

Table 2
Summary Results for the Central Luzon Forecast (No ASF Scenario).

SARIMA Model	AIC	BIC	WPE
(1,1,2)(0,1,1)	1466.28	1471.06	4.13

autocorrelation is present in the data up to the specified lag.

Moreover, for Central Luzon with the ASF scenario, the model chosen was SARIMA (0,1,2) (0,1,1), with a MAPE of 10.12 and an AIC of 1804.08 (Table 4). The ACF plot in Fig. 2 shows that only one line exceeds the range, which indicates a favorable model. As shown in Table 5, the Box-Ljung test's p-value of 0.76 indicated white noise (or no autocorrelation).

Fig. 2 shows the resulting impact of INSPIRE in Central Luzon. If there was no INSPIRE project, zero pig production would be forecasted by 2022Q1. The INSPIRE project resulted in additional pig production of 137,269 live weight metric tons (MT) in 2022 and 180,186 MT in 2023. Furthermore, it is interesting to note that in a no-ASF scenario, the industry would have continued a consistent trend of pig production volume with gradual annual growth. With the sweeping impact of ASF in 2019 and more drastically in 2020, the trend was interrupted, and a sharp drop resulted. Upon the implementation of the INSPIRE program, the decreased trend of pork production was terminated, and a gradual increase was recorded, showing a sign of recovery of production levels that could be attributed to government policy and limited interventions to the industry through INSPIRE. Despite a slow growth rate, the growth from the period of 2022–2024 has been consistently increasing, showing the program's impact on local industry momentum in terms of pork production. Central Luzon was the Philippine's leading pork-producing region with the presence of commercial-scale pig farms and a robust presence of backyard livestock. Being known as home to the country's voluntary capital, pork consumption is also high in the area. Medium-scale and cooperative-based farms, though at present, are inferior in size and capacity in Central Luzon compared to corporate pig farms. INSPIRE extent only to these medium-scale and cooperative farms given the limitation provided by relevant implementing guidelines. There is an appreciable impact, as seen in the data, but this did not cause significant recovery back to pre-ASF levels in the time-period covered, because of the slower recovery efforts on the side of the commercial and corporate pig farms that are not covered by direct INSPIRE interventions.

3.2.2. CALABARZON

For the CALABARZON no-ASF scenario, the model chosen was SARIMA (1,0,0) (0,1,1), with a MAPE of 5.48 and an AIC of 1416.73 (Table 3). The ACF plot in Fig. 3 shows that only one line exceeds the

range, which indicates a favorable model. As shown in Table 7, the Box-Ljung test's p-value of 0.57 indicated white noise (or no autocorrelation).

Moreover, for the CALABARZON with ASF scenario, the model chosen was SARIMA (0,1,0) (0,1,0), with a MAPE of 5.34 and an AIC of 1662.22 (Table 6). The ACF plot in Fig. 4 shows that only one line exceeds the range, which indicates a favorable model. Based on the data in Table 6, the Box-Ljung test's p-value of 0.04 indicated white noise (or no autocorrelation).

Fig. 7 shows the resulting impact of INSPIRE on CALABARZON. If there was no INSPIRE project, zero pig production would be forecasted by 2022Q1. The INSPIRE project resulted in additional pig production of 137,269 live weight MT in 2022 and 231,204 MT in 2023. In a similar way to that of Central Luzon, the entire industry of CALABARZON was not to continue a trend of annual increase in growth but was halted with the ASF problem evident in the significant fall of production volume in the year 2020. Recovery efforts, both in the private and government sectors resulted in a slight increase in production in 2021. This trend, however, was punctuated with subsequent decrease in 2021 and the early half of 2022 because of episodes of resurgence of ASF outbreaks in the region. From there, with the INSPIRE intervention in full swing, the data show that there has been a gradual increase in pork production levels. The CALABARZON swine industry landscape is more diversified by small and medium-scale pig farms and cooperative-based swine farming. Although there exist commercial-level farms in the region, farmer-level production is prominent widely because of a stronger entrepreneurial drive and capability among individuals and cooperatives. Despite the risk of ASF and refection, this entrepreneurial drive enabled farmers and cooperatives to manage the risk and proceed with the recuperative efforts as most of their livelihoods only center on pig farming. This regional landscape allowed INSPIRE to cater to more small hold, medium-scale and cooperative-based farmers that led to a

Table 3
Box-Ljung Test for the Central Luzon Forecast (No ASF Scenario).

Lagrange	p-value
0.21	0.77

Table 4
Summary Results for the Central Luzon Forecast (with the ASF Scenario).

SARIMA Model	AIC	BIC	WPE
(0,1,2) (0,1,1)	1804.08	1813.79	10.12

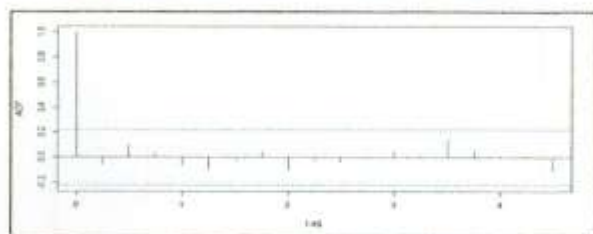


Fig. 2. ACF Results for the Central Luzon Forecast (No ASF Scenario).

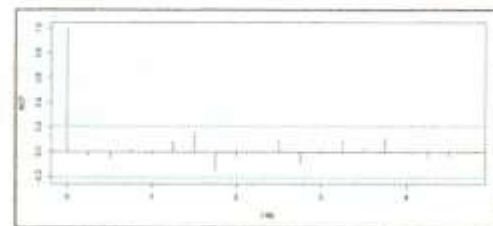


Fig. 3. ACF Results for the Central Luzon Forecast (with the ASF Scenario).

Table 5
Box-Ljung Test Results for the Central Luzon Forecast (with the ASF Scenario).

Lagrange	p-value
0.24	0.79

higher growth in pig production levels within the period covered in this study. This landscape may, could be the reason for punctuated growth patterns because of specific occurrences of outbreaks given the industry heterogeneity capabilities among non-commercial entries.

3.2.2. Davao region

For the Davao region no-ASF scenario, the model chosen was SARIMA (0,0,0) (0,1,0), with a MAPE of 5.74 and an AIC of 1270.58 (Table 8). The ACF plot in Fig. 8 shows that only one line exceeds the range, which indicates a favorable model. As shown in Table 8, the Box-Ljung test's p-value of 0.42 indicated white noise (0,1,0).

Moreover, for the Davao region with the ASF scenario, the model chosen was SARIMA (2,0,0) (0,1,0), with a MAPE of 2.92 and an AIC of 1466.55 (Table 11). The ACF plot in Fig. 9 shows that only one line exceeds the range, which indicates a favorable model. Based on the data in Table 11, the Box-Ljung test's p-value of 0.92 indicated white noise

(or no autocorrelation).

Fig. 11 shows the resulting impact of INSPIRE in the Davao Region. The forecasted Davao (with the ASF scenario) data does not differ much from the Davao PSA pig production data. Part of this reason could be that the region was hit by ASF later than the 2 other regions and in a lower extent. Compared with the Davao PSA data, the 9th quarter of 2022 and 2023 of Davao (with the ASF forecast) are even higher, which suggests that INSPIRE negatively affected pig production during those two quarters. The ASF scenario outlined the supposed trajectory of growth of pig production in the Davao region. While the actual industry production managed to narrow the difference that ASF caused in terms of pig production levels vis-a-vis the annual expected level, it is important to note that the characteristics of the swine industry in the Davao region are dominated by smallholder farms compared to the number of medium-scale and commercial-scale pig farms. According to Table 11, compared to the budget allocations that Central Luzon (PWF 4 billion or \$34.8 million) and CALABARZON (PWF 60 million or

Table 6
Summary Results for CALABARZON Forecast (No ASF Scenario).

SARIMA Model	AIC	BIC	WPE
(1,0,0) (0,1,1)	1416.73	1425.44	5.48

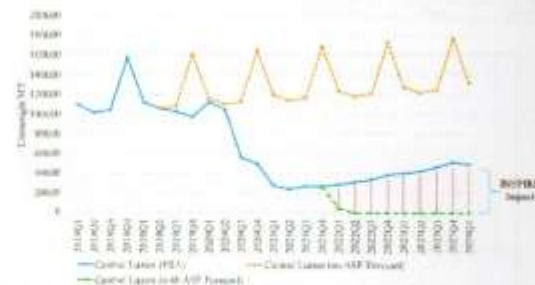


Fig. 4. Central Luzon Pig Production Comparison, 2018–24. Source: PSA, authors' estimation.

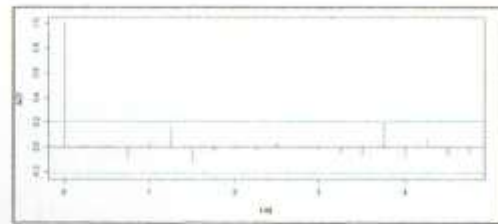


Fig. 9. ACF Results for the Davao Region Forecast (with the ASF scenario).

Table 13
Box-Ljung Test Davao Region Forecast (with the ASF scenario).

Expected	Actual
0.01	0.00

Including the Philippines (DDBusiness, 2011). Compared with Central Luzon and the Davao Region, CALABARZON produced the highest returns. However, the Davao region produced the lowest returns compared with the other two regions.

3.4 Investment requirements for pig production

Since 2020, there has been a need for Philippine pig production to increase by 6.6 % annually from 2023 to 2028 to return to the pre-ASF production of 2.32 million MT (Eq. (1)). If the current NLP budget is maintained, there is even a decline in pig production because of demand and existing ASF cases. The 2000Q1 to 2020Q4 datasets were used to forecast the remaining quarters until 2028Q4, with the assumption that the DA-NLP budget will remain at its current level. The chosen model was SARIMA (1, 1, 1)(1, 1, 1), with a MAPE of 2.22 and an AIC of 2093.69 (Table 14). The ACF plot in Fig. 10 shows that only one line exceeds the

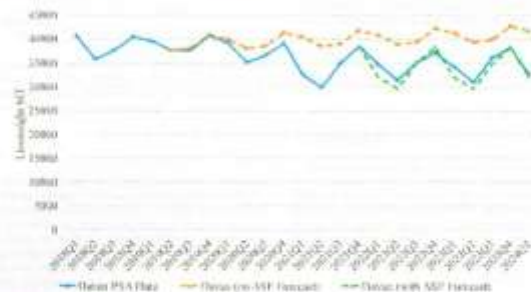


Fig. 10. General Pig Production Comparison. Source: PSA, authors' calculation.

Table 14
Budget Allocation for the 3 Regions under ISPPRF (in PHP).

Region	2023 Budget	2024 Budget	2024 Budget	Total
Central Luzon	906,800,000	696,264,000	0	1,603,064,000
CALABARZON	116,000,000	202,000,000	30,000,000	348,000,000
Davao Region	202,000,000	180,000	0	202,180,000

Source: DA-NLP Budget Utilization Report.

Note: The PHP to US exchange rate for 2023–2023 is 1:54.16 = \$1.

Table 15
Summary Analysis of ISPPRF Projects for 2023–2028.

Indicator	2023 ISPPRF	2023 ISPPRF
Central Luzon CR Ratio	20	26
Central Luzon NPL 12 %	16020.076,064,004	16,074,620,111,104
CALABARZON CR Ratio	10	12
CALABARZON NPL 22 %	16702,000,000,000	16,700,000,000,000
Davao Region CR Ratio	2	0
Davao Region NPL 12 %	16701,000,000,004	16,700,000,000,004

Note: The PHP to US exchange rate for 2023–2023 is 1:54.16 = \$1.

range, which indicates a favorable model. Based on the data in Table 17, the Box-Ljung test's p-value of 0.48 indicated white noise (or no autocorrelation).

Surprisingly, a downward trend in pig production was detected. If the current actual budget level in the appropriation program is maintained in the medium term, pig production may decline. This suggests that a higher budget (2–3 times the current budget) for the implementation of pig industry regulations is needed to arrest the decline in pig production and achieve the goal of the ISPPRF program of restoring the Philippine pig sector to its pre-ASF 2018 levels.

Table 16 shows the investment requirements needed to increase pig production by 6.6 % annually until the end of 2028, when production potentially returned to pre-ASF 2018 levels. It is not necessary that the government shoulder all the investment requirements. The government merely acts as an enabler of the environment and as a jump starter to increase the confidence of domestic and backyard farmers in pig production. Pig farmers will raise pigs voluntarily if it is profitable for them to do so, as evidenced by the incentive band of Adam Smith in the words of Williams (2005).

The private sector has shown a willingness to invest towards pig production recovery as noted in the following announcements. In southern Philippines (Iligan City, Misamis Oriental), Malabuta Agro Ventures Corporation is partnering with Denmark-based Danbred to establish a large-scale genetic pig farm aimed at supporting reproductive effects in the value of ASF in the form of PRRS6 infection or 546.3 million (Pangasinan, 2021). JC AgriSystems, Inc., forged a long-term agreement with PIC, the global leader in swine genetics, to establish and operate a broiler nucleus and multiplier farm in Nueva Ecija (Central Luzon) that will significantly expand PIC's capacity to produce broilers locally and disseminate genetic improvement to help Filipino farmers rebuild and improve the industry (PIC, 2023). Apart from these announcements in the media and those seeking endorsement for tax holidays from Department of Agriculture (DA), it is difficult to analyze private sector investments.

The unique contribution of this paper to the academic literature is the use of the time series SARIMA modeling to measure the impact of pig production program (ISPPRF) in the Philippines by modeling data set with ASF and without ASF scenarios. A related study is that of the Three-Correlated Model used by Jhon et al. (2017a) when they modeled

Table 16
Summary Results for the Philippine Pig Production Forecast (with the ASF scenario).

SARIMA Model	AIC	MAPE
(1,1,1)(1,1,1)	2093.69	2.22

Philippine chicken and beef prices with and without PRRS. Other related studies are that of Looi (1995) and Williams (1997). Looi (1998) used regression analysis, a form of time series, to ascertain the effects of the program in the Philippines. Williams (1997) applied multiple time series analysis which are applied to government decision inputs (the number of dredge and fill permits issued) and environmental quality outputs (the number of water quality violations) to test the use of multiple time series methods on natural resources policy (also separate genetic management).

4. Lessons learned

The lessons learned from this study can be summarized in two. First, time series ARIMA modeling using the with ASF and without ASF scenarios are useful in evaluating the impact of a pig population program. Previous research tools used DA to the animal population impacts are based on and simple forecasting. With the use of time series ARIMA modeling in R, the method becomes scientific. And lastly, these regions that produce minimal results even with infusion of financial resources. Given the budget limitation and the need for the prudent use of taxpayers' money, the results of this paper will be very useful to the broader international academic community. Based on the results of Fig. 4, Fig. 7, Fig. 10, and Table 14, there are large economic impacts caused by the pig population program in Central Luzon and CALABARZON, whereas it is minimal only in the Davao Region. This means that, whether there is ISPPRF (or any pig population program) or not, the Davao Region will continue to produce pigs. Since the government is always scrutinizing the subject and is always looking for value for taxpayers' money, there is a need to identify scarce government resources where it will have the greatest economic impact or gain. In designing future pig population programs, the Philippine government should first measure the economic impacts of the 2023–2023 ISPPRF on the 13

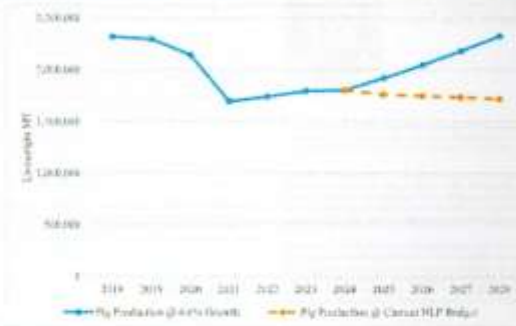


Fig. 11. Pig Production Higher Budget vs. Current NLP Budget. Source: PSA, authors' forecast.

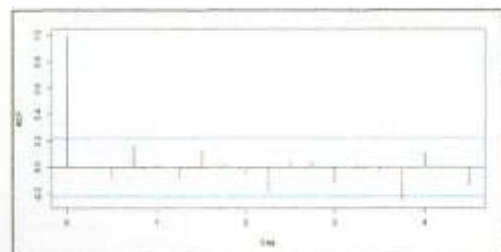


Fig. 5. ACF Results for CALABARZON Forecast (w/ ASF Scenario).

Table 7
Box-Ljung Test for CALABARZON Forecast (w/ ASF Scenario).

Statistic Model	ML	RM	RMSE
Engelend	0.9406		
LM	0.07		

Table 8
Summary Results for the CALABARZON Forecast (with the ASF Scenario).

Statistic Model	ML	RM	RMSE
(2,0,0) (0,1,0)	1463.07	1094.43	5.34

₱14.8 million) received during the period for the implementation of the INSURE Program from 2002 to 2006. Davao Region received a loan amount (PMP42 million, or ₱4.2 million) which despite yielding output, we note that significant in making significant effects to the cumulative regional production levels. This could also be attributed to the continuous and active reorganization initiatives of the private sector in the medium-scale and commercial level farms.

Government programs for livestock industries that particularly involve subsidies, grants, and assistance to domestic producers and stakeholders prove to have an impact on the production levels of livestock in the medium term. In Turkey, government funding for

livestock supports markedly increased over a 10-year period, from a mere 0.2 % of the total agricultural budget in 2004–2007 to 2014 (Liu et al., 2016). This, in turn, resulted to a steady increase in the inventory and production volume of major livestock and poultry commodities in the same period, with growth extending even up to the 2030 forecasts (Yener & Dincel, 2021). Similarly, government support for farm investments and activities fostered significant farm growth in the livestock sector of Austria, which showed a rapid response to investments in terms of inventory and farm size (Cachope & Koozevnik, 2012). Among highly self-sufficient countries is beef, as analysis of government expenditures and support revealed their effectiveness in maintaining and improving production levels (Dimitrakou et al., 2011).

Programs, and thereby the expenditures allocated by governments for the development of the livestock sector, result in appreciable gains in terms of growth in inventory and production levels. Like the impact of the INSURE program of the Philippine government, the pig sector benefited to a certain extent, particularly in meeting massive losses in

Table 9
Box-Ljung Test Results for the CALABARZON Forecast (w/ ASF Scenario).

Statistic Model	ML	RM	RMSE
Engelend	0.9406		
LM	0.04	0.04	

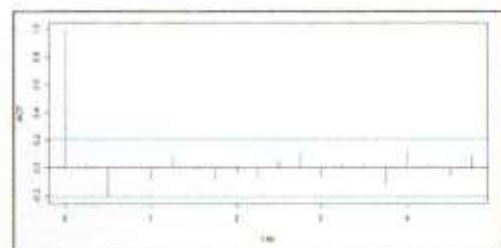


Fig. 6. ACF Results for CALABARZON Forecast (w/ ASF Scenario).



Fig. 7. CALABARZON Pig Production Comparison. Source: PIA, author's calculation.

Table 10
Summary Results for the Davao Region Forecast (w/ ASF Scenario).

Statistic Model	ML	RM	RMSE
(2,0,0) (0,1,0)	1270.92	1095.02	5.74

pig inventory and pig-based livelihood. The responsiveness of the sector to developmental interventions initiated by the government, demonstrated through production levels, is worth noting in the assessment of the success and effectiveness of the program. This study is the first to analyze the impact of a government program for pig industry reorganization in response to the damage of ASF that directly aided pig farmers with the introduction of farm structural reform. More than just policy and regulations, the Philippine government's approach to agricultural development heavily involves direct production support to farmers, and this is the main strategy of the INSURE Program. The study, therefore, is in a unique position to provide analysis of industry capabilities of government budget allocations and production support inputs directly given to farmers.

3.3. Economic analysis

Table 11 shows the calculation methodology for the OIA and NPV. Since this is a 5-year measurement of the effects, it is easier to calculate as opposed to multiyear effects (OIA can also be used for multi-year calculations depending on the purpose). The discount rate used was 12 %. The Asian Development Bank has recommended the use of a 10–12 % discount rate for the appraisal of projects in its Member States.

Table 11
Box-Ljung Test for the Davao Region Forecast (w/ ASF Scenario).

Statistic Model	ML	RM	RMSE
Engelend	0.9406		
LM	0.04	0.04	

Table 12
Summary Results for the Davao Region Forecast (with the ASF Scenario).

Statistic Model	ML	RM	RMSE
(2,0,0) (0,1,0)	1466.82	1472.01	5.62

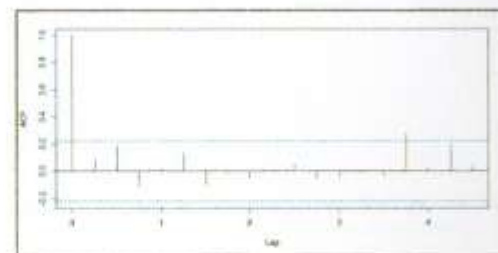


Fig. 8. ACF Results for the Davao Region Forecast (w/ ASF Scenario).

L.B.B. Abo et al.

where an agent or Program Coordinator oversees or manages planning, management, two other, implementation, and a third (A2027-2034). In addition to the agent role as an educator, he also serves as the site coordinator and manager of the Volunteer Site (Kawabata et al. 2016).

Education and Program Planning (E2) (2025, 2027)

Josephine M. Mays, MA, is a PhD candidate in Agricultural Extension and Application at Dakota State University in Aberdeen, South Dakota, and is Assistant Lecturer in Extension at the University of Northern Iowa. Her research interests include agricultural extension, agricultural education, and agricultural development. She teaches courses in program planning, extension-based research, and extension-based evaluation, and she develops materials and strategies for rural extension.

Table 1
Sequences for primers for qPCR analysis of target genes.

Target gene	Forward sequence	Reverse	Size of amplified product (bp)
RGF10	NC_049021.1 F: GGAGTGTAGGCTTACAA R: CAGCTCTTCTGTCCTCT		80
IT2	NC_049021 F: TGGGACAGCCCTGAGAA R: CAGCAAGTATACAGGAT		100
IT2	NC_049021.1 F: ACCGACCTGCTGCTC R: CAGGCTCTCTCCGACG		80
IT4	NC_049021.0.1 F: GGTGAGCTTATGCTTC R: GCTGCTTTCCTCCCTCT		77
IT6	NC_049021.0.1 F: CACTGCTTCTGCTTTC R: GCTGCTTTCCTCCCTCT		77
GAPDH	NC_049024.4 F: CCGGACAGCTTCTCT R: AGCCTTCTGAGTCTGACT		80

cDNA equivalent to 5 µg of starting total RNA was used as template for each real-time PCR reaction. Forward and reverse primers (Table 1) were created with Primer Express v3.0.1 software (Applied Biosystems, Foster City, CA, USA) and confirmed for target identity with Primer Blast from the National Center for Biotechnology Information (NCBI) [Ye et al., 2017]. Real-time PCR System, Rotor Light Cycler96 was used for qPCR with a 3-min denaturation, followed by 10 cycles of 95 °C for 15 s, 62 °C for 20 s and 72 °C for 25 s. High-resolution melting analysis was performed for each run.

Reactions were set up with PowerUpTM SYBR Green Master Mix (Applied Biosystems, Foster City, CA, USA) following the manufacturer's instructions. The amount of cDNA equivalent to 5 µg of starting total RNA was used as template for each real-time PCR reaction.

For relative gene expression studies, one housekeeping gene (glyceraldehyde 3-phosphate dehydrogenase (GAPDH)) was amplified alongside the target genes (IT2, IT4, IT6, IT8, and RGF10). Quantitative PCR was done in duplicate for each sample.

2.4. Statistical analysis

Using the Pfaffl method [Pfaffl, 2001] which requires primer efficiency for both gene of interest (GOI) and housekeeping genes using a standard curve and its cycle threshold (Ct) values and accounts for differences in primer efficiencies during the fold change calculation. The relative quantification of the target gene was determined by comparing the expression levels of GAPDH as a reference gene and GOI of HSP90, IT2, IT4, IT6, and IT8 with Spring season water as the calibrator. The standard deviation (SD) of the relative gene expression was determined by calculating the mean value of the breed population's gene expression ratio per GOI, which then use to calculate the individual deviation within the breed. InqPCR version 2017.0 software [BioRad] et al., 2017) was used to calculate primer efficiency. Further, because the substantial variations in relative gene expression levels makes finding statistically significant differences between groups more difficult, Ct values with less fluctuations were employed to detect statistically significant differences between groups. Ct value data were analyzed by mixed Analysis of variance (ANCOVA) with a general linear model (GLM) with repeated measurement in SPSS Version 25 (IBM Corp., Armonk, NY, USA) with breed, sex, season, breed*sex, breed*season, sex*season, and breed*sex*season as the mixed design factors. Mauchly's test was used to decide the assumption of sphericity in the repeated measurement, and Levene's test was based on the median for the equality of error variances within season measurement. Further, a post-hoc multiple comparison test was done using the Tukey test for breed and sea-

son as the factors. Visualization of the data was done using GraphPad Prism version 6.0.0 for macOS (GraphPad Software, San Diego, CA, USA). The results are shown as the mean ± SD. A difference with p < 0.05 was determined as statistically significant.

3. Results

3.1. Climatological conditions

The climatic data during the sampling day in each season of the year and the calculated temperature humidity index (THI) is presented in Table 2. The THI in all sampling days were within the thermoneutral zone, except for summer season which was 79.99

3.2. Ct value

The gene expression of all GOI (Table 2) in this study were significantly different (p < 0.05) in each season, showing the dynamic gene expression according to seasonal variation. The interaction between the season, breed, and sex were identified in IT2 gene expression (p < 0.05), indicating the changes in gene expression in each season were not equivalent in each breed and sex groups. According to the between-group test, it was found that there were significant differences in gene expression (p < 0.05) in each breed group across the season for IT2 and IT8A genes.

3.3. Relative gene expression

The relative expression was calculated using the Pfaffl method [Pfaffl, 2001] and is presented in Table 3 and visualized as Fig. 1 (A-F). The spring season was considered as a thermo-neutral season and used as a calibrator.

For RGF10, relative gene expression with different pools was observed, typing for Hungarian indigenous Tsigai (1.039 ± 0.226) and White Dörper (1.079 ± 0.441), summer (7.494 ± 11.032) for Hungarian Merino. In all GOI in this study, the lowest expression relative to the spring season was found in the autumn.

An increasing gene expression trend was identified for IT2 in the summer season. The highest relative gene expression means of IT2 were found in the summer season for White Dörper (17.370 ± 5.848) and Hungarian indigenous Tsigai (6.299 ± 0.812), but for Hungarian Merino, the highest expression was identified in the winter season (2.553 ± 2.136).

For the two fat-like merger genes, IT4 and IT6A, in spring and autumn, the gene expression tend to be low and a higher relative gene expression was observed in winter and summer. Both genes were observed to be overexpressed during the summer time in all breeds, with the mean relative gene expression value of 12.053 ± 11.018, 3.517 ± 2.720, and 14.263 ± 13.477 for IT4B in Hungarian indigenous Tsigai, Hungarian Merino, and White Dörper, respectively. While for IT6B were 5.347 ± 5.481, 7.497 ± 4.567, and 5.910 ± 8.131, respectively.

Besides that, for IT8A, the highest expression was observed in the summer for Hungarian Merino (15.204 ± 10.950) and White Dörper (14.263 ± 12.477), but not for Hungarian indigenous Tsigai, which was observed in the winter (2.283 ± 1.817). But still, a lower expression was observed during the thermoneutral seasons; spring and autumn.

4. Discussion

The health and well-being of animals are determinate factors in achieving maximum productivity. The seasonal stress is one of the major components in achieving this optimum point in livestock farming, as has been proven by many previous findings

Table 2
The THI in each sampling season.

Season	Date	Time	Temperature (°C)	Relative humidity (%)	THI
Spring	20/04/2020	12:00-12:30	19.37	62.04	54.84
Summer	12/06/2019	12:00-12:30	32.80	34.00	79.99
Autumn	19/11/2020	12:00-12:30	7.94	30.24	46.18
Winter	22/01/2020	12:00-12:30	5.51	60.30	26.95

Table 3
Mean and standard deviation of Ct value of RGF10, IT2, IT4, IT6, and IT8 of Hungarian indigenous Tsigai, Hungarian Merino, and White Dörper in each season of the year.

Gene	Season	Ct value (Mean ± SD, deviation)		White Dörper	Overall
		Hungarian indigenous Tsigai	Hungarian Merino		
RGF10	Summer	22.340 ± 0.473	22.508 ± 0.276	22.506 ± 0.620	22.516 ± 0.282**
	Autumn	24.875 ± 1.037	24.411 ± 1.266	25.000 ± 0.750	24.950 ± 0.262*
	Winter	22.962 ± 1.240	22.973 ± 0.955	23.534 ± 0.597	22.957 ± 0.674*
	Spring	22.821 ± 0.024	22.402 ± 0.106	22.888 ± 0.061	22.074 ± 0.767*
	Overall	22.486 ± 1.127*	22.987 ± 0.219*	22.752 ± 1.084*	22.084 ± 1.763
IT2	Summer	33.493 ± 1.581	32.897 ± 1.055	32.122 ± 1.096	32.883 ± 1.080*
	Autumn	34.215 ± 0.751	34.137 ± 0.834	35.145 ± 0.441	34.488 ± 0.752*
	Winter	28.869 ± 1.686	28.592 ± 0.975	28.135 ± 0.444	28.864 ± 1.119*
	Spring	29.755 ± 0.681	29.474 ± 0.251	28.110 ± 0.117	29.481 ± 0.862*
	Overall	30.421 ± 1.792*	30.290 ± 0.791*	28.436 ± 1.052*	30.048 ± 0.874*
IT4	Summer	26.947 ± 1.250	26.494 ± 1.007	26.117 ± 1.287	26.497 ± 1.021*
	Autumn	27.744 ± 1.270	25.276 ± 0.202	25.498 ± 0.248	26.205 ± 1.147*
	Winter	24.195 ± 1.214	24.113 ± 1.210	23.837 ± 0.243	24.026 ± 0.862*
	Spring	24.462 ± 0.779	24.443 ± 0.147	24.015 ± 0.057	24.189 ± 0.716*
	Overall	25.778 ± 2.024*	24.602 ± 1.011**	24.439 ± 1.202*	25.281 ± 1.330*
IT6	Summer	24.601 ± 0.751	24.183 ± 0.540	23.802 ± 0.020	24.192 ± 1.000*
	Autumn	26.613 ± 1.076	25.691 ± 1.521	25.732 ± 0.623	26.041 ± 1.040*
	Winter	23.603 ± 2.275	23.787 ± 1.328	22.483 ± 0.403	23.296 ± 0.627*
	Spring	24.489 ± 0.767	23.617 ± 0.717	23.393 ± 0.319	23.447 ± 0.591*
	Overall	24.975 ± 1.467*	24.481 ± 1.187*	23.768 ± 0.267*	24.274 ± 1.265*
IT8	Summer	25.572 ± 1.185	25.030 ± 1.404	25.071 ± 1.534	25.246 ± 1.130*
	Autumn	25.951 ± 0.805	24.830 ± 1.326	24.340 ± 0.071	25.081 ± 1.062*
	Winter	27.840 ± 5.558	25.430 ± 1.150	25.048 ± 0.517	27.397 ± 1.998*
	Spring	27.081 ± 0.767	25.951 ± 0.626	25.567 ± 0.217	27.198 ± 0.618*
	Overall	24.838 ± 1.708*	24.439 ± 1.016*	24.550 ± 0.579*	24.391 ± 1.008*
GAPDH	Summer	26.240 ± 2.238	25.911 ± 2.008	25.083 ± 1.106	26.012 ± 2.016*
	Autumn	22.872 ± 1.181	23.470 ± 0.718	23.962 ± 0.367	22.701 ± 0.916*
	Winter	21.831 ± 1.734	21.787 ± 1.386	21.187 ± 1.082	21.516 ± 0.861*
	Spring	21.902 ± 0.197	21.716 ± 0.266	21.640 ± 0.054	21.643 ± 0.614*
	Overall	23.240 ± 2.551*	22.818 ± 2.211*	22.858 ± 2.570*	22.857 ± 2.280*

SD: Standard deviation; *: different expression showed significant difference (p < 0.05).

Table 4
Relative gene expression calculation with 2019 method [20] of RGF10, IT2, IT4, IT6, and IT8 of Hungarian indigenous Tsigai, Hungarian Merino, and White Dörper in each season of the year, relative to spring season as a calibrator.

Gene	Season	Relative gene expression (Mean ± SD)		
		Hungarian indigenous Tsigai	Hungarian Merino	White Dörper
RGF10	Summer	0.177 ± 0.210	0.404 ± 11.013	0.027 ± 0.010
	Autumn	0.217 ± 0.176	0.319 ± 0.181	0.011 ± 0.001
	Winter	0.001 ± 0.000	4.487 ± 0.042	0.124 ± 0.008
	Spring	1.000 ± 0.000	1.000 ± 0.000	1.076 ± 0.001
	Overall	0.209 ± 0.223	1.975 ± 1.340	17.391 ± 0.889
IT2	Summer	0.219 ± 0.176	0.204 ± 0.210	0.001 ± 0.001
	Autumn	0.020 ± 0.010	0.001 ± 0.001	0.001 ± 0.001
	Winter	1.124 ± 0.576	1.000 ± 0.204	5.900 ± 6.417
	Spring	12.053 ± 11.018	3.517 ± 2.720	14.263 ± 13.477
	Overall	0.380 ± 0.210	0.380 ± 0.074	0.474 ± 0.176
IT4	Summer	2.027 ± 1.738	1.018 ± 1.737	1.004 ± 1.005
	Autumn	1.029 ± 0.261	0.079 ± 0.231	1.107 ± 0.433
	Winter	6.179 ± 0.001	13.284 ± 10.048	14.263 ± 13.477
	Spring	6.028 ± 0.000	6.124 ± 0.225	6.414 ± 0.116
	Overall	3.283 ± 1.817	3.414 ± 0.212	7.048 ± 3.005
IT6	Summer	1.000 ± 0.000	1.000 ± 0.000	1.167 ± 0.000
	Autumn	2.287 ± 1.461	1.467 ± 0.287	0.168 ± 0.108
	Winter	0.257 ± 0.001	0.842 ± 0.153	0.129 ± 0.000
	Spring	1.501 ± 1.818	0.017 ± 0.023	0.008 ± 1.000
	Overall	1.061 ± 0.406	1.271 ± 1.076	1.186 ± 0.737

SD: Standard deviation.

Original article

Hungarian indigenous Tsigai, a promising breed for excellent heat tolerance and immunity

Patri Kusuma Astuti^{a,b}, Zoltán Bagi^a, Lilla Bodrogi^a, Tímea Pintér^a, Gabriella Skoda^a, Roland Fajardo^{a,c}, Szilvia Kiszta^{a,d}^aFaculty for Aquaculture, Fisheries and Fisheries Biology, University of Debrecen, Debrecen 4002, Hungary^bNational School of Aquaculture, University of Debrecen, Debrecen 4002, Hungary^cDepartment of Animal Biotechnology, Budapest University of Agriculture and Life Sciences, 1056, Hungary^dDepartment of Agriculture - Bureau of Animal Industry, 1000, Davao, Davao City, Philippines

ARTICLE INFO

Article history:
Received 5 June 2023
Revised 13 July 2023
Accepted 22 July 2023
Available online 2 August 2023

Keywords:
Gene expression
Heat stress
Heat shock
Tsigai
HSP70
IL10

ABSTRACT

The adverse effects of climate change on sheep farming have become more noticeable in recent decades. Effective efforts have been made to mitigate the complex relationship between heat tolerance, animal health, and productivity, also to identify a resilient and economically suitable breed for selection that can be resilient to future climate change conditions. Using quantitative real-time polymerase chain reaction (qRT-PCR), we observed the seasonal variation in the expression of several important genes related to heat stress and immunity (HSP70, IL10, TNF α , IL1 β , and IL6) in three of the most widely kept sheep breeds in Hungary: the indigenous Tsigai, Hungarian Merino, and White Dorper. We found that the seasonal stressor affected the relative gene expression of all genes in this study. Notably, the Hungarian indigenous Tsigai was the most robust breed adapted to the Hungarian continental (hot summers, cold winter) environment, with excellent thermotolerance and immunity. Furthermore, despite reliance from heat stress in the summer, Hungarian Merino maintained their robust immune system well throughout the year.

© 2023 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Sheep (this genus) have been thought to be one of the most robust livestock that has inhabited a wide range of geographical areas since their domestication due to their exceptional adaptability to various types of diets and tolerance to adverse climatic conditions (Cogan et al., 2009; Sawaya and Nawayseh, 2019). However, in these constantly changing climate conditions, sheep are facing additional stresses (Shah, 2001; Nagy et al., 2021; Mchicha et al., 2022). These challenges have significant economic consequences

as well. Farmers of one of the major sheep exporters, New Zealand, for example, have been facing impact of climate change for more than a decade, which unfortunately decreases the profitability of their production (Wright et al., 2009). Heat stress seriously endangers reproduction of certain sheep breeds, and thus appears as a notably risk for efficient sheep management (Van Weeren et al., 2022).

Many aspects of cellular behaviour must change in response to environmental stressors, one of them is the changes in gene expression, which are a fundamental component of stress reactions (de Nadal et al., 2017), as are changes in metabolism, cell cycle progression, protein homeostasis, cytoskeletal architecture, vesicular trafficking, and enzymatic activity modification, which require more energy expenditure and may compromise animals' growth potential and productive capacity (Richter et al., 2016). Excessive heat exposure triggers multiple cellular responses and induces transcription and translation of several genes and modifications in protein synthesis, such as increased expression of heat shock proteins (HSPs), which function as intracellular chaperones in heat-stressed animals, preventing protein and cell damage (Haugen et al., 2018).

* Corresponding author.

E-mail address: astuti@debrecen.hu (P.K. Astuti), szilvia.kiszta@udm.ac.ph (S. Kiszta), roland.fajardo@udm.ac.ph (R. Fajardo), zoltan.bagi@udm.ac.ph (Z. Bagi), tima.pinter@udm.ac.ph (T. Pinter), lilla.bodrogi@udm.ac.ph (L. Bodrogi).

Peer review under responsibility of King Saud University.



Production and hosting by Elsevier

<https://doi.org/10.1016/j.sjbs.2023.10197>

1750-5052/2023 The Author(s). Published by Elsevier B.V. on behalf of King Saud University.

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

The expression of HSP is a specific sign of animal response to intense environmental stress. On the basis of their molecular size and similarity in amino acid sequence, HSPs are divided into multiple families (Morley, 2006). Genetic and biochemical research indicates that the 70-kDa heat shock protein (HSP70) family assists in the folding of proteins during translocation in the cytoplasm of both prokaryotic and eukaryotic cells and is primarily found in cytosol and nucleus. These are constitutively expressed and induced by various of HSP70 that are activated by stress stimuli (Larsen, 2004). Its expression is a possible indicator of an animal's capacity to adjust to intense environmental stress; a strong association exists between the development of ARS and the induction of thermotolerance through the inhibition of stress kinase activity (Dang et al., 2014).

Besides the disruption in HSP cellular mechanism, the alteration in immune-related gene regulation to either compromise or enhance immune cell activity is also induced during heat shock (Bachmann et al., 2016). Interleukin-10 (IL10) is a major immunoregulatory molecule released by macrophages, regulatory T cells, dendritic cells and certain epithelial cells (Mallikarajasinga et al., 2020). In sheep, IL10 has been linked to susceptibility to various diseases, such as *Fraxiparis pastus* virus (FPV) (Wang et al., 2018), bluetongue virus (BTV) (Kawthar-Vasquez et al., 2015), and *Haemaphysalis contorta* infection (Giles, 2012). Another one is the Toll-like receptors (TLRs), an extensively studied immune-related gene expressed on numerous cell types, including mononuclear cells and tissue immune cells (Liu, 2009; Zhang et al., 2021). TLRs are a collection of evolutionarily conserved pattern recognition receptors (PRRs) that can detect a variety of pathogen-associated molecular patterns (PAMPs) from a wide range of microorganisms and activate innate immune responses (Dewery and McWhorter, 2007) and are found to be essential for host resistance to gastrointestinal parasitic infection (Borawski et al., 2016) and Classical Swine Fever (García-Martínez et al., 2022).

With the threat of unrelentably climate change and its harmful effects, effort are constantly made to thoroughly comprehend how environmental conditions generated some coping mechanisms in the animal's body. Many studies has reported the seasonal effect on heat-stress related gene expression in livestock (Singh et al., 2014; Acharya et al., 2018; Kumar et al., 2018). Given the scarcity of research on studies of seasonal gene expression in sheep, this study aims to describe the seasonal changes of relative expression level of the following heat stress and heat stress-related genes: HSP70, IL10, TNF α , IL1 β , and IL6 in three sheep breeds raised in Hungary today (Hungarian indigenous Tsigai – indigenous breed in Central-Eastern-European; Merino – worldwide spread sheep breed, its dispersal started in the 18th century in Hungary; White Dorper – insular origin, was introduced in Hungary 10 years ago), with the goal of assessing the adaptability of the three breeds to Hungarian temperate climatic conditions, also to contribute with valuable knowledge on biological response to seasonal stressors in sheep.

2. Materials and methods

2.1. Sample collection and location

Total blood sample was initially collected from 24 animals (12 ewes and 12 rams) from three different breeds: Hungarian Merino, Hungarian indigenous Tsigai and White Dorper, four rams and four ewes per breed. All three breeds represent the major breeds raised in Hungary. Hungarian indigenous Tsigai is a natural breed, belonging to the Tsigai group indigenous to the Eastern and Central Europe. The Hungarian Merino is the result of Merino breeding

in Hungary beginning in the late 17th century and continuing into the 18th century, when it began to be crossed with local breeds. White Dorper is a relatively new breed in Hungary reported from South Africa. All animals included in the study were relatively the same age (2 to 3 years old), body weight (mean: 45 to 55 kg; range: 65 to 75), and in excellent health condition with no physical and anatomical abnormalities. The animals were kept in the Károlyi Experimental Station of Animal Husbandry of the University of Debrecen, located at 127 m above sea level (47.58° N and 21.58° E). The average annual maximum and minimum ambient temperature ranges from -7.5 to 28.0 °C with annual precipitation of 550 to 600 mm. The sampling was done in four different seasons in 2018–2020: spring (April), summer (August), autumn (November), and winter (January). All animals received the same management during the experimental period. The breeds were kept and fed together throughout the year, segregated by sex in sheep shed technology, which included an enclosure. The exception was the autumn breeding season, when they were kept in barns. All animals are fed ad libitum a diet which consisted of hay + fodder 0.4 kg/sheep/day, with free access to clean water. Fodder contained 50% corn, 50% hay. During lambing, ewes receive an extra 1 kg of alfalfa hay, 4 weeks before insemination and during insemination, they were additionally given alfalfa silage 1 kg/sheep/day. Selection tick blocks were available to the animals for 265 days.

Due to the lengthy research period, several animals had to be culled (died or removed from the farm) from the target individuals during implementation. In spring, all 24 samples were collected in summer 21 animals (1 Hungarian indigenous Tsigai ewe, 1 Hungarian indigenous Tsigai ram, and 1 Merino ram died); in autumn 17 animals (2 Merino ewes died and 2 White Dorper ewes removed from the farm), and in winter 15 animals (1 Merino (20 died) and 1 Merino ram was removed from the farm). At the end of the research period, 15 animals had completed four-season sampling: Hungarian Merino (2 ewes and 1 ram), Hungarian indigenous Tsigai (3 ewes and 3 rams), and White Dorper (2 ewes and 4 rams). In every peak season of the year, about 5 ml blood sample was obtained from the jugular vein of the same animals in Trispan® blood DNA Tube (Applied Biosystems) and preserved at -30 °C until further analysis.

2.2. Climatological data

The climatic conditions during the sampling day were recorded at every hour. The THI was calculated for each sampling day using the following equation by (Muller et al., 2009). The severity of heat stress in livestock is typically rated using the THI, which ranges from 6 (no stress) to > 64 (extreme heat stress), categorized as follows: no stress (<67), mild (68–74), moderate (75–78), severe (79–83), and extreme (>84) (Izquierdo-Baila et al., 2011).

$$THI = (0.8 \times T_{air}) + \left(\frac{RH}{100} \right) \times (T_{air} - 14.4) + 46.4$$

T_{air} : Dry bulb temperature (°C), RH: Relative humidity (%).

2.3. Quantification of gene expression level using qRT-PCR

The total RNA was isolated from 3 ml of total blood using Trizol reagent RNA Isolation Kit (Applied Biosystems, USA) following the manufacturer's instructions and treated with DNase (Qiagen, catalog number: 79256).

RNA quality and quantity assessment were done using a NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). 100 ng total RNA was reverse transcribed into cDNA with specific primers (Table 1) by the qPCRHD DNA Synthesis Kit (PCR Biosystems, London, United Kingdom). The amount of

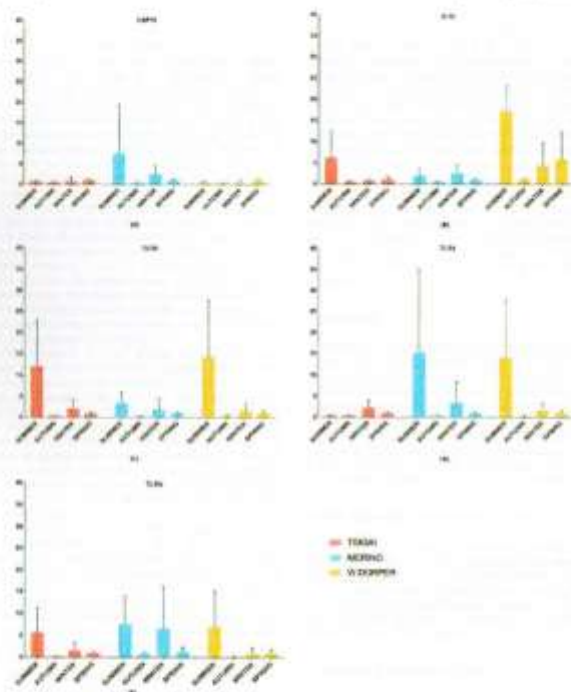


Fig. 5. Bar graph of relative gene expression of (A) HSP70, (B) IL6, (C) IL10, (D) SIRT6 and (E) TIMP1 in each month of the year with the spring months as the reference. The X-axis is the relative gene expression in arbitrary units of the year. The Y-axis is gene expression for each different breed in the study. Different colours represent different breeds: red for Hungarian indigenous Tsigai, blue for Hungarian Merino, and yellow for White Dorper.

(Lara and Montagnani, 2012; Hudson et al., 2018). The seasonal variation shows temperature, humidity, rainfall, and daylight problems across the year, which may affect the livestock's homeostasis directly through changes in physiological and biological processes, also behaviour adjustment (Mariani et al., 2018; Rothberg et al., 2019). Understanding the livestock's seasonal adjustment is critical for successful and profitable livestock farming, especially in areas where climate change is the most pressing issue, exposing animals to temperatures beyond their comfort zones and compromising their production ability.

The thermal comfort zone for sheep varies among breeds, which correlates to their morphological characteristics facilitating the body's heat exchange with the environment, for example the coat colour and characteristics or the physical characteristics of the hair-

coat (Farré et al., 2014). This morphological diversity in livestock provides a potent system for genetic dissection of traits with complex inheritance, which also demonstrates their adaptation to their environment and is an indication of natural selection favouring greater diversity (Boyer et al., 2010). In this study, the three most common sheep breeds in Hungary were observed: the Hungarian indigenous Tsigai, the commercial breed with a long history or crossbreeding, the Hungarian Merino, which both are cold-tolerant breeds; and the South African commercial breed, White Dorper, which is a breed with a high tolerance to heat. All of the breeds in this study have a dominating white coat colour, with both the Hungarian indigenous Tsigai and Hungarian Merino having long wool and White Dorper having short wool. The aim was to see if there is any difference in adaptability to the Hungarian environ-

ment between the three different breeds, although all of them have been acclimatised to it as these breeds have been widely kept in Hungary.

Generally, sheep have an upper critical between 25 and 31 °C, depending on factors such as breed, age, and physiological state. Heat stress occurs when the effective temperature of the environment is higher than the upper critical temperature (Waggoner et al., 2010). To assess livestock productivity responses to climate change, the simplest method is to use the THI, which is calculated by combining the average temperature (°C) and relative humidity (%) of a given location (Kotchková et al., 2021). It is undeniable, however, that THI may not entirely portray the thermal environment in which animals live because it does not take into consideration such factors as wind velocity, solar radiation, the shade provided to animals, or the availability of water. According to the THI calculation of each sampling season, almost-severe heat stress was observed in the summer season (THI = 79.89), which generated some gene expression changes inside the sheep body, explained as follows.

Thermal acclimation and adaptation in small ruminants are inevitably correlated with increased HSP concentrations. The HSPs have been the core of molecular responses in livestock to heat stress, but its stress tolerance mechanism is complex and not fully understood. With high HSP concentrations, cells can respond to heat stress better, while low levels of HSP make them more vulnerable (Gaut et al., 2018; Jay et al., 2020). As it was observed in this study, Hungarian Merino, the cold-tolerant breed with a high susceptibility to heat stress, the relative gene expression of HSP70 was upregulated in the summer, indicating heat-stress-induced HSP70 expression. As explained by Almeida et al. (2018) and Hoque et al. (2018), a relatively higher HSP70 gene expression level suggests a greater need to protect the conformation of proteins on the cells from heat stress damage such as denaturation and aggregation. A previous study on goats showed HSP70 gene is essential in goat environmental stress tolerance and adaptation and its expression increases during heat stress (Boseppur et al., 2014; Anshana et al., 2015) along with a mechanism to protect proteins from degradation and facilitates their refolding, thereby enhancing cell survival (Danga et al., 2014).

The expression pattern of the HSP70 gene is species- and breed-specific, for goats, as shown by research by Ramirez et al. (2014), possibly due to variations in thermal tolerance and adaptability to different climatic circumstances. They also confirmed that constitutive expression of HSP70 gene is more remarkable in Indian cold-adapted goats (Gadhvi and Chappu) than in heat-adapted goats (Sohani and Barhani). The spike of HSP70 expression due to heat stress in other livestock has also been reported, e.g. cattle (Sharma et al., 2017) and buffalo (Vadav et al., 2021). In comparison, the Hungarian indigenous Tsigai and White Dorper (DP70) expression in this study showed a small range of relative gene expression during the season of the year.

The Hungarian indigenous Tsigai, as expected, has good adaptability to the Hungarian environment (Gágyardi et al., 2006) and Kuzay et al. (2010, 2011, 2015) explained that the Hungarian indigenous Tsigai sheep is an ancient breed that has its origin traced to Asia Minor, located in modern-day Turkey. This breed was introduced to Hungary during the latter half of the 1700s. The breed has gained importance in Hungary owing to its resistance and capacity to adapt to harsh climates and the breed has a historical significance, contributing to the country's agricultural heritage and economy. While the White Dorper has a better thermal tolerance due to its advantageous morphological characteristics such as short hair, thin skin, and a low number of hair follicles per unit area that help in combating heat stressors by facilitating heat dissipation (Gauthey, 2013) without necessitating them to make physiological regulation such as increasing heart

rate and respiration rate to help to release the internal heat or reducing food intake and body weight to decrease the endogenous heat production (Kuzay et al., 2017). Many findings have proven their extraordinary thermoregulation and resistance to heat stress (Almeida et al., 2011; Jay et al., 2020).

The colour and characteristics of an animal's coat can have a significant impact on its ability to acclimate to changing temperatures. Wool not only has significant economic value, but also according to a review by Anon et al. (2022) acts as a protective barrier that helps regulate body temperature by preventing heat loss in cold environments and blocking excessive heat gain in hot environments. Nevertheless, the thickness and type of wool can also impact an animal's ability to regulate its body temperature. For instance, wool that is too thick may have a lower thermoregulatory capacity due to the insulation effect of the wool, which isolates body heat from escaping. In contrast, animals with thinner wool may have improved thermoregulation due to air stability within the fleece, which reduces heat loss via convection (McManus et al., 2020). The heritability of wool traits is moderate to low as they are influenced by multiple genetic and environmental factors. The wool-growing process in the epidermis involves intricate coordination among numerous genes and cell types, such as fibroblast-anchoring enzyme 2 (FA2H) (HRT23) and Keratin-like GTPase binding protein 2 (KRTAP2) which play crucial roles in keratinocyte differentiation and cell proliferation (Zhuo et al., 2021). Filaggrin (FFL) genes which regulate hair follicle morphogenesis and cycling (Zhuo et al., 2017), also keratins (KRTs) and keratin-associated protein (KRTAP) which also plays important role in epithelial cell's stress response and apoptosis (Dehghan et al., 2016). The genetic and cellular mechanisms underlying the relationship between wool characteristics of sheep and their thermal adaptation remains obscure and complex. The incomplete understanding of the complexities of these biological processes contributes to a continuing lack of clarity on the subject.

Apart from its direct role in molecular protection against heat stress (intercellular function), the external HSP70 also plays a vital role in the immune system. One is by signalling immune cells against invading pathogens via increased neutrophils and macrophages (Dybdahl et al., 2003), in collaboration with heat pattern recognition molecules like toll-like receptors (TLR) through the pathogen-associated molecular pattern (PAMP) to activate immune response (Kuzay et al., 2018). The other is through the intervention of intracellular inflammatory signalling pathways, such as the production of interleukin-10 (IL10), the known anti-inflammatory and immunosuppressive cytokine (Hoque et al., 2012).

Heat stress causes an imbalance in the immune system by shifting the adaptive immune function from the normal cell-mediated to humoral immunity, which is a shifted contribution to the poor health that results from it. Heat stress has been shown to reduce sheep's immunity, making them more susceptible to disease (Soghli et al., 2016; Shi et al., 2020). It is widely thought that heat-stress-induced glucocorticoids impair immunological homeostasis via altered cytokine production. However, the specific mechanism is unknown (Raghu et al., 2010). In this study, the expression of immune-related genes (IL10, IL6, IL18, and TIMP) was also studied to reveal the relationship between heat stress and immune deprivation in sheep.

The expression of IL10 was significantly different in each season and was not equalised in each breed, in White Dorper, it was over-expressed in the summer, as was the case in Hungarian indigenous Tsigai but with a lower peak. However, in Hungarian Merino, peak expression was seen only during the winter and seasonal variation was minimal. The previous study on various cattle breeds, Kuzay Fries (Kuzay et al., 2016), Sahiwal cows (Gautwal et al., 2021), and Jersey (Shi et al., 2020) showed a higher IL10

