Scientific Research Publishing

Pharmacology & Pharmacy, 2025, 16(10), 341-352
https://www.scirp.org/journal/pp
ISSN Online: 2157-9431
ISSN Print: 2157-9423

Assessment of Histopathological and Blood Biochemical Profiles in Dairy Cattle Treated with an Experimental Intramuscular Fasciolicide

Froylán Ibarra-Velarde[1], Rosa Arias-García[1], Yolanda Vera-Montenegro[1*], Miguel Flores-Ramos[2], Gerardo Leyva-Gómez[3], Alicia Hernández-Campos[3]

[1]Departamento de Parasitología, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Ciudad de México, México
[2]Unidad Mérida, Escuela Nacional de Estudios Superiores, Universidad Nacional Autónoma de México, Ucú, Mexico
[3]Departamento de Farmacia, Facultad de Química, Universidad Nacional Autónoma de México, Ciudad de México, México
Email: *vemonty@yahoo.com

How to cite this paper: Ibarra-Velarde, F., Arias-García, R., Vera-Montenegro, Y., Flores-Ramos, M., Leyva-Gómez, G. and Hernández-Campos, A. (2025) Assessment of Histopathological and Blood Biochemical Profiles in Dairy Cattle Treated with an Experimental Intramuscular Fasciolicide. *Pharmacology & Pharmacy*, **16**, 341-352.
https://doi.org/10.4236/pp.2025.1610018

Received: August 22, 2025
Accepted: October 11, 2025
Published: October 14, 2025

Abstract

The aim of this work was to determine whether intramuscular administration of injectable fasciolicide fosfatriclaben, in a single dose at 6 mg/kg in dairy cattle, produces adverse reactions manifested through blood biochemical and histopathological profiles, especially in organs involved in drug metabolism. For the study, nine out of ten Holstein-Freisian dairy cows were treated, leaving one cow untreated as a control. On days 0, 7, 14, and 28 post-treatment, liver, kidney, and injection site samples were taken for histopathology, as well as blood samples for biochemical analysis. The results showed no histopathological changes, and the analyte values in biochemical tests remained within the reference range, suggesting the compound has a favorable safety profile.

Keywords

Fasciola Hepatica, Triclabendazole, Fosfatriclaben, Adverse Drug Reaction

1. Introduction

Cattle parasites represent a constant and serious threat to the development of the livestock industry. Worldwide, cattle producers incur millions of dollars in losses due to reduced zootechnical performance of animals, due to the presence and action of the trematode parasite *Fasciola hepatica*, causing an adverse economic impact. The magnitude of losses justifies parasite control measures to protect profits

and improve animal welfare [1]. Triclabendazole (TCBZ) is the most widely chosen fasciolicide due to its efficacy against both the adult and juvenile flukes [2] [3]. It is a compound with very poor water solubility, like all benzimidazoles [4]. Fosfatriclaben is a new prodrug, a TCBZ derivative, highly soluble and stable in water. Initial studies on its fasciolicide efficacy have presented positive results [5]-[7].

Since it is a new formulation, the available information is limited and requires testing through various clinical trials before commercialization. This is to document its benefits or adverse reactions in organs most susceptible to so-called drug-induced injuries, and also to guarantee its pharmacological effectiveness, as well as its level of safety and quality [8]-[13]. The effectiveness of a compound is determined both by its efficacy (its ability to produce the results for which it was designed), as well as by the absence of adverse reactions, or how severe or tolerable these are [14].

An adverse drug reaction (ADR) is a harmful or unpleasant reaction resulting from use of medicine in an individual, which predicts danger of future administration and justifies prevention, treatment, dosage alteration, or product withdrawal [15] [16]. ADRs can be minor, moderate, severe, or lethal; immediate, short-term, long-term, or permanent; and can be due to the active substance, contaminants, or excipients [11] [15] [17]. ADRs are a major source of morbidity, mortality, and increased healthcare costs [18] [19].

To protect public health, all reactions observed during the development and testing of a medicinal product for food-producing animals should be considered significant, and the diagnosis of association with ADRs should be specified [9] [20] [21]. Any organ or system could be affected by pharmacodynamic interactions [22]; however, the liver and kidneys are particularly susceptible to ADRs due to their extensive involvement in drug metabolism and excretion [23]-[26]. The impact of ADRs on livestock farming lies in productive profitability. Subacute or chronic alterations due to kidney or liver damage compromise the productive performance of animals [22].

Histopathological and biochemical analyses are diagnostic alternatives for detecting whether the administration of a medication produces alterations in liver or kidney functions, or in other organs, such as the muscle and skin at the injection site. Histopathology allows determination of the presence or absence of alterations in sampled organs [12] [27]; and abnormal values in blood biochemical could indicate some pathology or enzymatic problems due to medication administration, whether or not accompanied by clinical signs [15] [27] [28]. In live animals, the inoculation site may be inspected for any reaction such as pain, swelling, redness, heat, loss or decrease in function, and any other visible or palpable changes [12].

2. Materials and Methods

2.1. Study Location

The study was carried out on a farm located in Tulancingo, Hidalgo, central Mexico.

2.2. Animals

The study involved 10 clinically healthy Holstein-Friesian dairy cows, males, with an average initial weight of 50 kg each. They were born and housed within the study location, and were fed under pasture conditions, alfalfa, and open water.

2.3. Experimental Compound

Experimental prodrug (fosfatriclaben) was synthesized and formulated by our research group in the Facultad de Química of the Universidad Nacional Autónoma de México (UNAM).

2.4. Treatments and Necropsy

On day 0, nine out of ten Holstein-Freisian dairy cows were treated with fosfatriclaben injected at 6 mg/kg intramuscularly, single dose; one cow remained as an untreated control. Euthanasia was carried out by qualified farm personnel in accordance with current health regulations [29], using captive-bolt stunning on days 0, 7, 14, and 28 post-treatment [30] [31]. The determination of these times was based on the efficient use of the animals, according to the principle of reduction alternatives (fewer animals, 3R) to maximize the information obtained per animal without compromising animal welfare, and thus potentially limiting or avoiding the subsequent use of other animals [32]-[34]. Therefore, in order to obtain the greatest number of experimental results with the fewest animals [35], sampling for this work was proposed within a cow group acquired for adjacent research on residues in edible tissues, which will be carried out by high-performance liquid chromatography at the established slaughter times.

2.5. Sample Collection and Evaluation

For histopathological processing of sectioned tissue samples for hematoxylin and eosin staining on slides, samples were collected from all cows. These 2 × 2 cm samples came from the liver, kidney, and injection site (femoral muscles of the hind limb); they were preserved in glass containers with 10% formalin in a 1:10 ratio [36]. On days 0, 7, and 14, two treated cows were sampled each time, and on day 28, the three remaining treated cows were sampled. The control cow was sampled on day 0. For biochemical analysis, blood samples were taken from the subset of animals scheduled for necropsy on days 0, 7, 14, and 28 post-treatment. Blood samples were collected from the jugular vein into heparinized Vacutainer tubes and centrifuged for 10 minutes at 3500 rpm, transferring plasma to 2 mL Eppendorf vials. The basic blood biochemical profile included glucose, blood urea nitrogen (BUN), creatinine, bilirubin, aspartate aminotransferase (AST), glutamate dehydrogenase (GDH), creatine kinase (CK), total protein, albumin, globulins, calcium, phosphorus, sodium, potassium, chloride, bicarbonate, GAP anion, strong ion difference (SID), and osmolarity. All samples were sent under refrigeration to the Departamento de Patología at Facultad de Medicina Veterinaria y Zootecnia (FMVZ-UNAM).

3. Results and Discussion

Histological sections of liver, kidney, skin, and muscle from the inoculation site, did not present evident pathological changes, neither in treated cows nor in the control. In the blood biochemical results (Table 1), no values were found outside of the reference range provided by the Departamento de Patología at FMVZ-UNAM. Statistical tests for dispersion were not performed to compare mean results due to the numerical restriction of using a single control animal.

Table 1. Blood biochemical report results of cows treated with fosfatriclaben at 6 mg/kg intramuscularly and of the untreated cow.

Analyte	Unit	Treated cows[a]	Control cow	Reference value
Glucose	mmol/L	3.77	4.2	2.34 - 8.27
BUN	mmol/L	5.03	6.2	0.4 - 6.8
Creatinine	mmol/L	81.89	74	45 - 128
Bilirubin	mmol/L	2.29	3.1	0 - 11.4
AST	U/L	60.56	88	4 - 116
GDH	U/L	18.00	21	<31
CK	U/L	218.00	291	<375
Total protein	g/L	63.78	70	46 - 80
Albumin	g/L	28.44	28	19 - 46
Globulins	g/L	35.33	42	27 - 50
Calcium	mmol/L	2.59	2.61	2.27 - 2.95
Phosphorus	mmol/L	2.06	2.13	1.61 - 3.80
Sodium	mmol/L	135.78	136	131 - 148
Potassium	mmol/L	5.13	4.7	3.9 - 5.5
Chloride	mmol/L	101.22	99	93 - 106
Bicarbonate	mmol/L	25.56	27	23.5 - 27
GAP anion	mmol/L	14.22	15	13 - 20
SID	mosm/kg	34.56	37	30 - 40
Osmolarity	mmol/L	271.11	270	270 - 300

UV-visible spectrophotometry, Dirui CS-T240, dcL-SEKISUI reagents, FMVZ, UNAM; [a]Averages; BUN: blood urea nitrogen. AST: aspartate aminotransferase. GDH: glutamate dehydrogenase. CK: creatine kinase. SID: strong ion difference.

These results suggest a favorable safety profile and confirm the results obtained in previous studies [5]-[7] [35], on the similarity that could characterize the new prodrug fosfatriclaben with respect to its precursor, TCBZ, in clinical safety terms,

since no histopathological or biochemical findings were observed that would allow us to interpret adverse drug reactions (ADRs) derived from its application. This research is part of a series of studies involving fosfatriclaben. The objectives include corroborating its high efficacy, high clinical safety, and, in general, establishing its full potential as a fasciolicide alternative in sheep and cattle.

Fosfatriclaben has appropriate characteristics to be administered parenterally (neutral pH, high solubility, and stability in aqueous solution), and so far no signs of pain at the animal inoculation site, side effects, or toxicity have been observed [5] [6] [35], which is corroborated in this research. Its intramuscular application facilitates administration to large groups of animals, in addition to requiring a lower dose compared to TCBZ (*per os*), and with similarly high fasciolicidal activity [6].

The parenteral administration exhibits several advantages, such as first-pass metabolism avoidance, better bioavailability, and reliable dosage. Compared with oral administration, the parenteral route has control over the dose and rate, thus generating more predictable pharmacodynamic and pharmacokinetic profiles. It is also a suitable way to administer medicines to animals that are weakened or have difficulty swallowing [37] [38]. Medications applied parenterally are absorbed more quickly compared to oral ingestion, meaning they have a faster onset of action. Because they do not undergo digestive processes in the gastrointestinal tract, they are metabolized differently, resulting in a stronger effect than oral medications [38].

Moreover, the intramuscular route (IM) also has other benefits such as a rapid and uniform absorption of the drug, especially in aqueous solutions; rapid onset of action (a few minutes) compared to that of the subcutaneous route (SC), due to muscle vascularization. It has efficacy and potency comparable to that of the intravenous drug delivery system, is highly effective for emergency scenarios, and a large volume of medicine can be administered compared to the SC route [39] [40]. Disadvantages of the oral route include the relatively slow onset of action, possibility of irregular absorption, destruction of acid-labile drugs in the stomach, low bioavailability of poorly water-soluble drugs, and some drugs may cause local irritation of the gastrointestinal mucosa [40].

One of the practical scopes of this injectable fasciolicide is its rapid application and, consequently, the reduction of animal stress in large-scale livestock management, reducing in turn the risk of injury among animals, operators, and veterinarians. The management and immobilization of sheep and cattle with slow flow can induce very high stress levels due to the high presence of cortisol [41] [42]. Stress, in addition to disrupting animal welfare, negatively affects the profitability and economic viability of livestock farming [43]. The stress response includes alterations whose consequences reduce zootechnical performance, such as changes in immune function and consequent increased susceptibility to disease, decreased feed intake and rumination, leading to weight loss, deterioration in meat quality, inhibition of oxytocin release, reduced fertility, and mortality [44]-[48].

Animals present more aggressive behaviors when the containment time is longer [49] [50], which may be related to long periods of exposure to extreme climates, invasion of individual space, interaction between submissive and dominant animals, or animals of different ages and sexes (biological stressors), as well as contact with unfamiliar operators within facilities [45] [50]-[53]. Delayed animal handling times are associated with increased reactivity, more undesirable behaviors, and a higher risk of accidents. The number of animals displaying stressful behaviors is closely related to handling time [45] [54] [55]. Likewise, there is a possibility that because it takes longer to handle each animal for oral treatments, the last animals in the group may perceive stress signals for longer, further reducing their flow rate. The emission of both behavioral and chemical stress signals increases the stress of the last animals passing through the handling chute [56]. Therefore, optimizing pharmacological presentations is critical for achieving clinical efficacy and safety [40].

Preclinical safety studies aim to fully characterize the potential actions of a therapeutic compound under development, including evaluation of toxicity in organs, to rule out adverse effects [11]. Anticipating the RAM profile allows us to activate strategies that minimize risks and, at the same time, maintain its favorable pharmacological properties [57].

The clinical features, patterns, and diagnostic criteria of drug-induced liver injury are well described in humans [11] [27], but not so well in animals . Some diagnoses of drug-induced hepatotoxicity and nephrotoxicity in animals are based on the interpretation of abnormal blood chemistry values and degenerative, inflammatory, or necrotic histopathological findings, whether or not accompanied by clinical signs [15] [18] [25] [27] [58]-[62]. Local reactions at the inoculation site, as well as hypersensitivity, are also common [62]-[64]. Ruminant antiparasitics are generally considered safe; however, potential ADRs need to be assessed for all new pharmaceutical compounds.

There are no reports yet of alterations in liver or kidney function tests, or hematologic indices, attributable to TCBZ in human clinical trials; and no evidence of dose-related toxicity has been observed in animals [65]. Fosfatriclaben, being a derivative of TCBZ, could present similarity in terms of its safety index [66]. Moreover, in previous research, fosfatriclaben has presented high fasciolicidal efficacy close to 100% in reducing eggs and adults of *F. hepatica* [5]-[7], too similar to that of the best commercial fasciolicides, including its precursor, TCBZ.

One limitation of this study was the inability to statistically compare the results due to the restriction of having only one animal as a control. Additional studies are recommended to confirm the safety of this compound.

4. Conclusion

In this study, it was confirmed that fosfatriclaben did not produce signs of pain or inflammation at the animal inoculation site, nor did it produce any histopathological or biochemical findings that could be interpreted as an ADR caused by this

experimental prodrug, suggesting a favorable safety profile. IM drug administration is faster than the oral route, resulting in fewer animals experiencing stressful behaviors, more efficient management, and a higher level of animal welfare and safety for animals, farmers, and veterinarians.

Acknowledgements

This research was carried out thanks to the PAPIIT IT200422-DGAPA-UNAM project.

Availability of Data and Material

All datasets are included in this manuscript.

Funding

This study was funded by Universidad Nacional Autónoma de México, grant number PAPIITIT200422, awarded to Ibarra Velarde.

Contributors

Conceptualization, investigation, methodology, project administration, validation: FIV, RAG, YVM, AHC. Data curation and formal analysis: RAG, MFR. Funding acquisition: FIV. Resources: FIV, MFR, AHC, GLG. Visualization and writing—original draft: FIV, RAG. Writing—review and editing: FIV, RAG, YVM, MFR, AHC.

Animal Research

The authors affirm that all procedures carried out in this work comply with the ethical standards of national and institutional guidelines on the care and use of animals. This experimental protocol was approved by the Comité Interno para el Cuidado y Uso de los Animales (CICUA) of the Facultad de Medicina Veterinaria y Zootecnia (FMVZ), Universidad Nacional Autónoma de México (UNAM), with protocol number 115.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Strydom, T., Lavan, R.P., Torres, S. and Heaney, K. (2023) The Economic Impact of Parasitism from Nematodes, Trematodes and Ticks on Beef Cattle Production. *Animals*, **13**, Article No. 1599. https://doi.org/10.3390/ani13101599

[2] Dalton, J.P. (1999) Fasciolosis. CABI Publishing Series.

[3] Boray, J.C., Crowfoot, P.D., Strong, M.B., Allison, J.R., Schellenbaum, M., Von Orelli, M., *et al.* (1983) Treatment of Immature and Mature Fasciola Hepatica Infections in Sheep with Triclabendazole. *Veterinary Record*, **113**, 315-317. https://doi.org/10.1136/vr.113.14.315

[4] Lindenberg, M., Kopp, S. and Dressman, J.B. (2004) Classification of Orally Administered Drugs on the World Health Organization Model List of Essential Medicines According to the Biopharmaceutics Classification System. *European Journal of Pharmaceutics and Biopharmaceutics*, **58**, 265-278. https://doi.org/10.1016/j.ejpb.2004.03.001

[5] Flores-Ramos, M., Ibarra-Velarde, F., Jung-Cook, H., Hernández-Campos, A., Vera-Montenegro, Y. and Castillo, R. (2017) Novel Triclabendazole Prodrug: A Highly Water Soluble Alternative for the Treatment of Fasciolosis. *Bioorganic & Medicinal Chemistry Letters*, **27**, 616-619. https://doi.org/10.1016/j.bmcl.2016.12.004

[6] Arias-García, R., Vera-Montenegro, Y., Flores-Ramos, M., Castillo, R., Hernández-Campos, A. and Ibarra-Velarde, F. (2020) Efficiency Comparison of Experimental Fosfatriclaben with Three Commercial Fasciolicides in Experimentally Infected Sheep. *Parasitology Research*, **119**, 2687-2693. https://doi.org/10.1007/s00436-020-06705-4

[7] Flores-Ramos, M., Leyva-Gómez, G., Rojas-Campos, T., Cruz-Mendoza, I., Hernández-Campos, A., Vera-Montenegro, Y., *et al.* (2024) Fosfatriclaben, a Prodrug of Triclabendazole: Preparation, Stability, and Fasciolicidal Activity of Three New Intramuscular Formulations. *Veterinary Parasitology*, **327**, Article ID: 110113. https://doi.org/10.1016/j.vetpar.2024.110113

[8] Holloway, K. and Green, T. (2003) Comités de farmacoterapia Guía práctica. Organización Mundial de La Salud, 155. http://apps.who.int/medicinedocs/es/d/Js8121s/

[9] Brumbaugh, G.W. (2001) Adverse Drug Reactions and Interactions in the Horse. *Veterinary Clinics of North America*: *Equine Practice*, **17**, 445-453. https://doi.org/10.1016/s0749-0739(17)30044-5

[10] Maddison, J. (1996) Adverse Drug Reactions: Report of the Australian Veterinary Association Adverse Drug Reaction Subcommittee, 1994. *Australian Veterinary Journal*, **73**, 132-136. https://doi.org/10.1111/j.1751-0813.1996.tb10005.x

[11] Consolini, A.E. and Ragone, M.I. (2017) Farmacodinamia general e interacciones medicamentosas. In Edulp. https://doi.org/10.35537/10915/67056

[12] Sánchez-González, M.J., Barbarroja-Escudero, J., Antolín-Amérigo, D. and Rodríguez-Rodríguez, M. (2013) Reacciones alérgicas a fármacos. *Medicine—Programa de Formación Médica Continuada Acreditado*, **11**, 1808-1818. https://doi.org/10.1016/s0304-5412(13)70531-6

[13] Garbi Novaes, M.R., Pastor, E., Garbi Novaes, A., Guilhem, D. and Cañete Vil-lafranca, R. (2015). Reacciones adversas en ensayos clínicos con nuevos fármacos conducidos en Brasil. Años 2000 y 2012. *Revista Médica Electrónica*, **37**, 18-29. http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S1684-18242015000100003

[14] Lynch, S.S. (2022) Eficacia y seguridad del fármaco. MANUAL MSD. https://www.msdmanuals.com/es-mx/professional/farmacología-clínica/conceptos-farmacoterapéuticos/eficacia-y-seguridad-del-fármaco

[15] Edwards, I.R. and Aronson, J.K. (2000) Adverse Drug Reactions: Definitions, Diagnosis, and Management. *The Lancet*, **356**, 1255-1259. https://doi.org/10.1016/s0140-6736(00)02799-9

[16] Laurence, D.R. and Carpenter, J.R. (1998) A Dictionary of Pharmacology and Allied Topics. 2nd Edition, Elsevier.

[17] Smith Marsh, D.E. (2023) Reacciones adversas a los fármacos. MANUAL MSD. https://www.msdmanuals.com/es-mx/professional/farmacología-clínica/reacciones-adversas-a-los-fármacos/reacciones-adversas-a-los-fármacos

[18] Rieder, M.J. (1994) Mechanisms of Unpredictable Adverse Drug Reactions. *Drug Safety*, **11**, 196-212. https://doi.org/10.2165/00002018-199411030-00005

[19] Montané, E. and Santesmases, J. (2020) Reacciones adversas a medicamentos. *Medicina Clínica*, **154**, 178-184. https://doi.org/10.1016/j.medcli.2019.08.007

[20] SENASICA (Servicio Nacional de Sanidad Inocuidad y Calidad Agroalimentaria) (2016). Criterios de funcionamiento de métodos analíticos. https://www.gob.mx/senasica/documentos/criterios-para-el-funcionamiento-aplicacion-e-interpretacion-de-metodos-analiticos?state=published

[21] SENASICA (Servicio Nacional de Sanidad Inocuidad y Calidad Agroalimentaria) (2017) Programa nacional de control y monitoreo de residuos tóxicos en bienes de origen animal, recursos acuícolas y pesqueros 2017 y resultados del 2016. https://www.gob.mx/cms/uploads/attachment/file/265356/Programa_Nacional_de_Control_y_Monitoreo_de_Residuos_T_xicos_2017.pdf

[22] Arrieta, D., Comerma Steffensen, S. and Zerpa, H. (2015) Adverse Drug Interactions in Bovine. In: Salomon, J., Romero, R., Arias, M. and Diaz, H., Eds., *XXIX Workshop on Beef Cattle*, UCV, 1-20.

[23] Cano Paniagua, A., Cifuentes Posada, L.M. and Amariles Muñoz, P.J. (2017) Toxicidad hepática causada por medicamentos: revisión estucturada. *Revista Colombiana de Gastroenterología*, **32**, 337-348. https://doi.org/10.22516/25007440.177

[24] Calderón, C., Guzmán, G., Sarmiento, J., Gómez, D., Joya, A., Ríos, L., Romero, J., Rosado, K. and Soler, J. (2011). Nefrotoxicidad inducida por medicamentos. *Médicas UIS*, **24**, 65-85. https://revistas.uis.edu.co/index.php/revistamedicasuis/article/view/2583/2905

[25] Davis, J.L. (2011) Recognizing and Treating Adverse Drug Reactions (Proceedings). Dvm360, 1-12. https://www.dvm360.com/view/recognizing-and-treating-adverse-drug-reactions-proceedings-0

[26] Martín-Jiménez, T. (2002) Farmacocinética I: Absorción y distribución/Metabolismo, excreción y modelos farmacocinéticos. In: Botana López, L.M., Martín-Jiménez, T. and Landoni, M.F., Eds., *Farmacología y terapéutica veterinaria*, McGraw-Hill, 3-4, 33.

[27] Liu, L. and Liu, X. (2019) Contributions of Drug Transporters to Blood-Brain Barriers. In: Liu, X.D. and Pan, G.Y., Eds., *Drug Transporters in Drug Disposition, Effects and Toxicity*, Springer, 407-466. https://doi.org/10.1007/978-981-13-7647-4_9

[28] Paul, J.W. (1977) Clinical Considerations Regarding Drug Interactions in the Bovine Patient. *The Bovine Practitioner*, **1977**, 9-14. https://doi.org/10.21423/bovine-vol1977no12p9-14

[29] Servicio Nacional de Sanidad, I. y C. A. (2011) Manual de Procedimientos para el Sacrificio Humanitario y la Disposición Sanitaria en Emergencias Zoosanitarias. https://www.gob.mx/cms/uploads/attachment/file/483431/Manual_de_procedimientos_para_el_sacrificio_humanitario_y_la_disposici_n....pdf

[30] CAMEVET (2014) XX Seminario sobre Armonización del Registro y Control de Medicamentos Veterinarios Ottawa, Canadá. 66.

[31] Palma, C., Godoy, C., Arboix, M. and Pérez, R. (2006) Determinación de residuos de abamectina-triclabendazol en tejidos bovinos. *Archivos de Medicina Veterinaria*, **38**, 265-271. https://doi.org/10.4067/s0301-732x2006000300011

[32] Balls, M. (2009) The Three Rs and the Humanity Criterion (An Abridged Version of The Principles of Humane Experimental Technique by W.M.S. Russell and R.L. Burch). FRAME, 131 p.

[33] Scientific Committees, G. (2024) Three Rs Principle (in Animal Experimentation). Comisión Europea. https://ec.europa.eu/health/opinions/es/primates-no-humanos/glosario/pqrs/principio-tres-erres.htm

[34] Kramer, M. and Font, E. (2015) Reducing Sample Size in Experiments with Animals: Historical Controls and Related Strategies. *Biological Reviews*, **92**, 431-445. https://doi.org/10.1111/brv.12237

[35] Arias-García, R., Ibarra-Velarde, F., Vera-Montenegro, Y., Flores-Ramos, M., Hernández-Campos, A. and Leyva-Gómez, G. (2025) Histopathology and Blood Biochemical in Sheep Treated with an Experimental Intramuscular Fasciolicide. *Veterinaria México OA*, **12**, 1-13. https://doi.org/10.22201/fmvz.24486760e.2025.1385

[36] CENASA (2025) Catálogo de servicios. Centro Nacional de Servicios de Diagnóstico En Salud. https://www.gob.mx/cms/uploads/attachment/file/981287/C001-13_Cat_logo_de_Servicios.pdf

[37] Desai, A. and Lee, M. (2008) Book Review: Gibaldi's Drug Delivery Systems in Pharmaceutical Care. *Annals of Pharmacotherapy*, **42**, 1149. https://doi.org/10.1345/aph.1L012

[38] Open Resources for Nursing (Open RN) (2021) Administration of Parenteral Medications. In: Ernstmeyer, K. and Christman, E., Eds., *Nursing Skills*, Chippewa Valley Technical College, 1-13. https://www.ncbi.nlm.nih.gov/books/NBK593214/

[39] Gutierrez, P. and Munakomi, S. (2025) Intramuscular Injection. StatPearls Publishing.

[40] Robinson, M., DeDonder, K.D. and Duncan, F. (2023) Routes of Administration and Dosage Forms of Drugs (Vol. 2023). MSD Veterinary Manual. https://www.msdvetmanual.com/pharmacology/pharmacology-introduction/routes-of-administration-and-dosage-forms-of-drugs

[41] Grandin, T. (1997) Assessment of Stress during Handling and Transport. *Journal of Animal Science*, **75**, 249-257. https://doi.org/10.2527/1997.751249x

[42] Grandin, T. and Shivley, C. (2015) How Farm Animals React and Perceive Stressful Situations Such as Handling, Restraint, and Transport. *Animals*, **5**, 1233-1251. https://doi.org/10.3390/ani5040409

[43] Odeón, M.M. and Romera, S.A. (2017) Estrés en ganado: Causas y consecuencias. *Revista Veterinaria*, **28**, 69-77. https://doi.org/10.30972/vet.2811556

[44] Manteca, X., Mainau, E. and Temple, D. (2013) Estres en animales de granja: Conceptos y efectos sobre la producción. *Farm Animal Welfare Education*, **1**, 2.

[45] Damián, M.Á., Aguirre, V., Orihuela, A., Pedernera, M., Rojas, S. and Olivares, J. (2020) Tiempo de manejo y algunas conductas relacionadas con el estrés al manejar grupos grandes o reducidos de ganado en mangas rectas. *Revista Mexicana de Ciencias Pecuarias*, **11**, 905-913. https://doi.org/10.22319/rmcp.v11i3.5127

[46] Breuer, K., Hemsworth, P.H., Barnett, J.L., Matthews, L.R. and Coleman, G.J. (2000) Behavioural Response to Humans and the Productivity of Commercial Dairy Cows. *Applied Animal Behaviour Science*, **66**, 273-288. https://doi.org/10.1016/s0168-1591(99)00097-0

[47] Sutherland, M.A. and Dowling, S.K. (2014) The Relationship between Responsiveness of First-Lactation Heifers to Humans and the Behavioral Response to Milking and Milk Production Measures. *Journal of Veterinary Behavior*, **9**, 30-33. https://doi.org/10.1016/j.jveb.2013.09.001

[48] Paranhos da Costa, M. J., Macedo de Toledo, L. and Schidek, A. (2013). Boas Pácticas de manejo, Vacinação. Ministério da Agricultura, Pecuária e Abastecimento.

[49] Burdick, N.C., Randel, R.D., Carroll, J.A. and Welsh, T.H. (2011) Interactions between Temperament, Stress, and Immune Function in Cattle. *International Journal of Zoology*, **2011**, Article ID: 373197. https://doi.org/10.1155/2011/373197

[50] Proudfoot, K. and Habing, G. (2015) Social Stress as a Cause of Diseases in Farm Animals: Current Knowledge and Future Directions. *The Veterinary Journal*, **206**, 15-21. https://doi.org/10.1016/j.tvjl.2015.05.024

[51] Fukasawa, M. and Tsukada, H. (2010) Relationship between Milk Cortisol Concentration and the Behavioral Characteristics of Postpartum Cows Introduced to a New Group. *Animal Science Journal*, **81**, 612-617. https://doi.org/10.1111/j.1740-0929.2010.00770.x

[52] Asres, A. and Amha, N. (2014) Effect of Stress on Animal Health: A Review. *Journal of Biology, Agriculture and Healthcare*, **4**, 116-121.

[53] Enríquez, D., Hötzel, M.J. and Ungerfeld, R. (2011) Minimising the Stress of Weaning of Beef Calves: A Review. *Acta Veterinaria Scandinavica*, **53**, Article No. 28. https://doi.org/10.1186/1751-0147-53-28

[54] Ceballos, M.C., Sant'Anna, A.C., Góis, K.C.R., Ferraudo, A.S., Negrao, J.A. and da Costa, M.J.R.P. (2018) Investigating the Relationship between Human-Animal Interactions, Reactivity, Stress Response and Reproductive Performance in Nellore Heifers. *Livestock Science*, **217**, 65-75. https://doi.org/10.1016/j.livsci.2018.08.001

[55] Ceballos, M.C., Sant'Anna, A.C., Boivin, X., Costa, F.d.O., Carvalhal, M.V.d.L. and Paranhos da Costa, M.J.R. (2018) Impact of Good Practices of Handling Training on Beef Cattle Welfare and Stockpeople Attitudes and Behaviors. *Livestock Science*, **216**, 24-31. https://doi.org/10.1016/j.livsci.2018.06.019

[56] Orihuela, J. and Solano, J.J. (1994) Relationship between Order of Entry in Slaughterhouse Raceway and Time to Traverse Raceway. *Applied Animal Behaviour Science*, **40**, 313-317. https://doi.org/10.1016/0168-1591(94)90071-x

[57] Sutherland, J.J., Yonchev, D., Fekete, A. and Urban, L. (2023) A Preclinical Secondary Pharmacology Resource Illuminates Target-Adverse Drug Reaction Associations of Marketed Drugs. *Nature Communications*, **14**, Article No. 4323. https://doi.org/10.1038/s41467-023-40064-9

[58] Davis, L.E., Scalley, R.D., Abbitt, L.E. and Forney, S.D. (1977) Pharmacologic Basis of Adverse Drug Reactions. *The Bovine Practitioner*, **1977**, 2-9. https://doi.org/10.21423/bovine-vol1977no12p2-9

[59] Brown, S.A. and Garry, F.B. (1988) Comparison of Serum and Renal Gentamicin Concentrations with Fractional Urinary Excretion Tests as Indicators of Nephrotoxicity. *Journal of Veterinary Pharmacology and Therapeutics*, **11**, 330-337. https://doi.org/10.1111/j.1365-2885.1988.tb00192.x

[60] Hsu H, W. and Ahrens, F.A. (2008) Drug Interactions and Adverse Drug Reactions. In: Hsu H, W., Ed., *Handbook of Veterinary Pharmacology*, Wiley-Blackwell, 461-470.

[61] McDuffie, J.E., Gao, J., Ma, J., La, D., Bittner, A., Sonee, M., Wagoner, M. and Snook, S. (2013) Novel Genomic Biomarkers for Acute Gentamicin Nephrotoxicity in Dog. *Open Journal of Molecular and Integrative Physiology*, **3**, 125-133. https://doi.org/10.4236/ojmip.2013.33018

[62] Arunvikram, K., Mohanty, I., Sardar, K.K., Palai, S., Sahoo, G. and Patra, R.C. (2014) Adverse Drug Reaction and Toxicity Caused by Commonly Used Antimicrobials in

Canine Practice. *Veterinary World*, **7**, 299-305.
https://doi.org/10.14202/vetworld.2014.299-305

[63] Miller, W.H., Griffin, C.E. and Campbell, K.L. (2012) Bacterial Skin Diseases. In: Miller, W.H., Griffin, C.E. and Campbell, K.L., Eds., *Muller and Kirk's Small Animal Dermatology*, 7th Edition, Elsevier, 191-192.

[64] Voie, K.L., Lucas, B.E., Schaeffer, D., Kim, D., Campbell, K.L. and Lavergne, S.N. (2013) The Effect of "Allergenic" and "Nonallergenic" Antibiotics on Dog Keratinocyte Viability *in Vitro*. *Veterinary Dermatology*, **24**, Article No. 501.
https://doi.org/10.1111/vde.12060

[65] McCarthy, J.S. and Moore, T.A. (2015) Drugs for Helminths. In: Bennett, J.E., Dolin, R. and Blaser, M.J., Eds., *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*, Elsevier, 519-527.e3.
https://doi.org/10.1016/b978-1-4557-4801-3.00042-4

[66] Wolff, K., Eckert, J., Schneiter, G. and Lutz, H. (1983) Efficacy of Triclabendazole against Fasciola Hepatica in Sheep and Goats. *Veterinary Parasitology*, **13**, 145-150.
https://doi.org/10.1016/0304-4017(83)90074-2