



Diagnostic Exercise
From The Latin Comparative Pathology Group*

Postvaccinal pyogranulomas in cattle

Carlos Eduardo Bastos Lopes^{1**}, Luana Ferreira Marques Cordeiro¹,
 Sheila Neves Carvalho de Oliveira², Felipe Pierezan¹, Leandro do Carmo Rezende³,
 Fabiana Galtarossa Xavier³, Roselene Ecco¹

¹Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil;

²Serviço de Inspeção Federal, Mato Grosso, Brazil;

³Laboratório Federal de Defesa Agropecuária de Minas Gerais, Pedro Leopoldo, Minas Gerais, Brazil.

**Corresponding author: carlosbastes@vetufmg.edu.br

Clinical History:

The carcasses of seven 30-month-old female cows slaughtered in a local abattoir for meat production were totally condemned during meat inspection due to disseminated gross abnormalities.

Gross Findings:

On internal examination, several skeletal muscles (pectoralis superficialis and profundus, transversus abdominis, diaphragm, obliquus internus abdominis, and rectus abdominis) and random areas of the peritoneal cavity had multifocal to coalescing white and firm nodules (1-3 cm in diameter). The nodules were firmly adhered (fibrous adherences) to the adjacent tissues and surface of portions of the forestomach serosa and splenic capsule (Figs. 1A, B, and C). On the cut surface of the nodules, there was a central accumulation of a friable and yellow exudate surrounded by a fibrous capsule (Fig. 1D). No external lesions were seen in any of the cows.

Follow-up questions:

- *Microscopic description*
- *Morphologic diagnosis*
- *Probable cause*
- *Differential diagnoses*



**The Diagnostic Exercises are an initiative of the Latin Comparative Pathology Group (LCPG), the Latin American subdivision of The Davis-Thompson Foundation and published in cooperation with the Brazilian Journal of Veterinary Pathology. These exercises are contributed by members and non-members from any country of residence.*

Consider submitting an exercise! A final document containing this material with answers and a brief discussion will be posted on the CL Davis website:

<https://davisthompsonfoundation.org/diagnostic-exercise/>



Davis-Thompson
 Foundation

Editor-in-chief for this Diagnostic Exercise: Claudio Barros

Associate Editor for this Diagnostic Exercise: Saulo Pavarini

Gross and microscopic images:

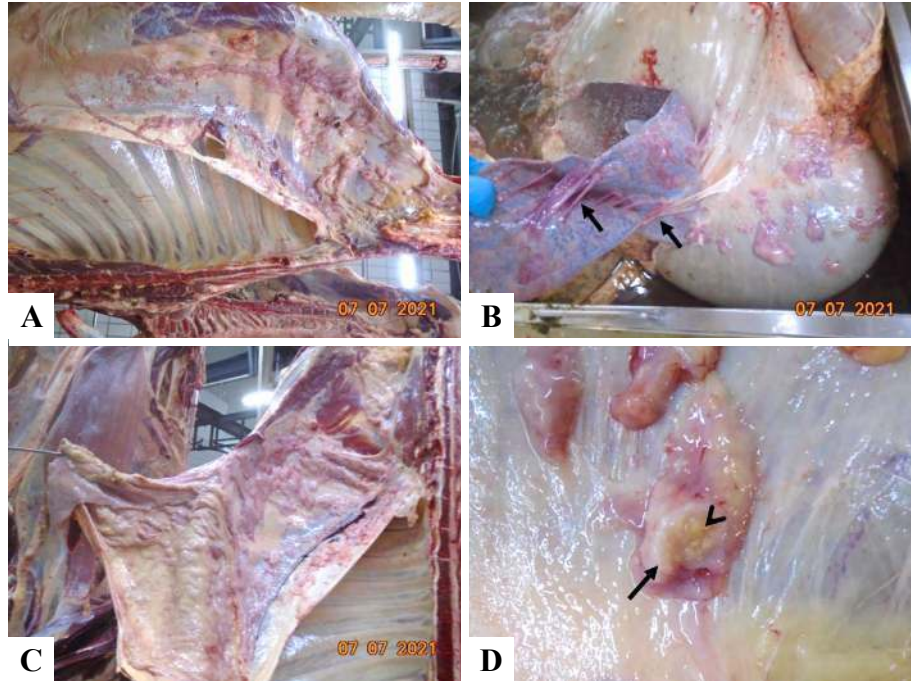


Figure 1. Postvaccinal pyogranulomas, cattle. (A) Diaphragm and transversus abdominis muscles. Multifocal to coalescing, white and firm nodules (pyogranulomas), surrounded by moderate amounts of fibrous tissue. (B) Splenic capsule and ruminal serosa. Multifocal to coalescing pyogranulomas with fibrosis and chronic adhesions (arrows). (C) Diaphragm, transversus abdominis, and obliquus internus abdominis muscles. Disseminated pyogranulomas with marked fibrosis. (D) Peritoneum. Cut surface of a pyogranuloma with caseous necrotic foci (arrowhead), surrounded by a fibrous capsule (arrow).

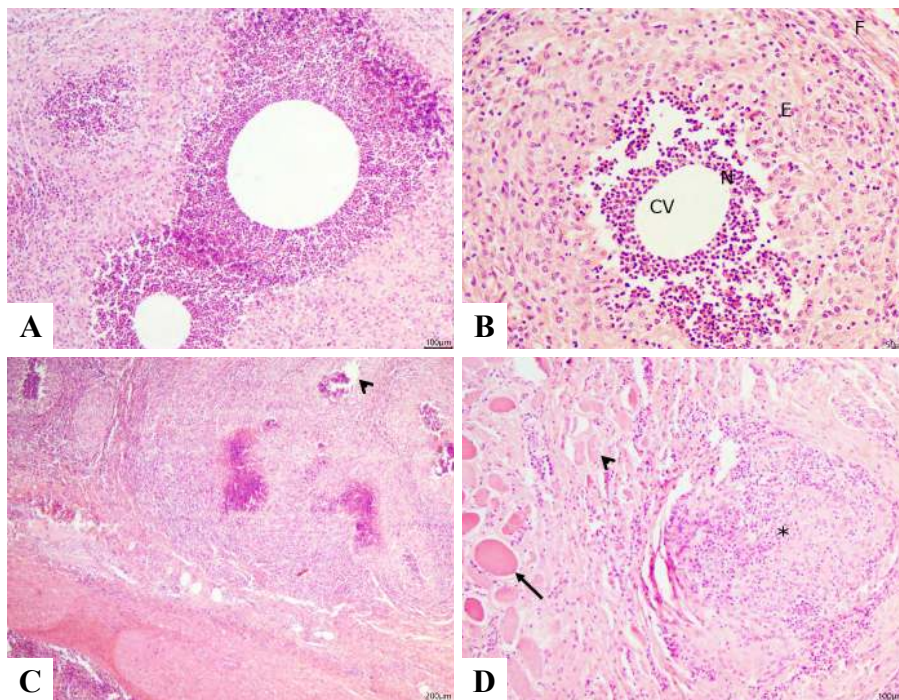


Figure 2. Postvaccinal pyogranulomas in abattoir cattle. A. Peritoneum, central clear spaces intimately associated with pyogranulomatous reaction, hematoxylin and eosin (H&E), 100x. B. Peritoneum, central clear vacuole (CV) surrounded by degenerate neutrophils (N), epithelioid macrophages (E) and halo of fibrosis (F), H&E, 200x. C. Spleen, extracapsular pyogranulomas with central clear vacuoles (arrowhead), H&E, 40x. D. Skeletal muscle, extensive pyogranulomatous reaction (asterisk), along with remaining disrupted (arrowhead) and hyalinized muscle fibers (arrow) interspersed by fibrous connective tissue, H&E, 100x.

Answers:**Microscopic description:**

Microscopically, throughout the analyzed tissue sections of the peritoneal and pleural membranes, there were multifocal to coalescing nodules with central clear and well-delimited vacuoles, ranging from 50-900 µm, surrounded by degenerate neutrophils, epithelioid macrophages, lymphocytes, plasma cells, and reactive fibroblasts (pyogranulomas) (Figs. 2A and B). The same inflammatory infiltrate was observed in areas of the splenic capsule (Fig. 2C) and skeletal muscles, with foci of degeneration and loss of rhabdomyocytes (Fig. 2D). There were rare multinucleated Langhans giant cells and mineral deposits at the center of some pyogranulomas. Additional histochemical techniques were applied to the lesions, including Grocott-Gomori's Methanamine Silver, Periodic acid-Schiff, Goodpasture, and Ziehl-Neelsen stains; however, no intralesional microorganisms were evidenced.

Morphologic Diagnosis:

Pleura, peritoneum, and skeletal muscles: marked, multifocal to coalescing, pyogranulomatous pleuritis, peritonitis, and rhabdomyositis with intralesional lipid vacuoles.

Probable Cause:

Iatrogenic administration of oily adjuvant vaccines.

Differential diagnoses:

- Disseminated tuberculosis.
- Non-tuberculous chronic peritonitis/pleuritis.
- Mesothelioma (gross differential only).

Discussion:

Post-injection pyogranulomas represent a diagnostic challenge if there is no history available at the time of the pathological analysis. We based the diagnosis of post-injection reaction on the typical microscopic findings and epidemiological outcomes.

Brazil is currently considered free of foot-and-mouth disease (FMD) with vaccination. Due to its extensive territory and the broad frontiers with nearby countries, it is difficult to control the introduction of new pathogens and outbreaks of highly contagious diseases. Since the last episode of FMD in 2006, the Brazilian Government has implemented important animal health policies, including massive cattle and buffalos' vaccination in all Brazilian states, except for Santa Catarina, which has the status of free FMD without vaccination (2). Oily vaccines against FMD must be applied subcutaneously or intramuscularly in the lateral cervical region (5), since this route facilitates the dissipation of the oily adjuvant (5). However, the route is associated with a higher local reaction. Improperly trained

personnel frequently contribute to the development of post-injection pyogranulomas (4, 9).

Due to the proximity of the vaccine application site to the spinal cord, post-vaccinal granulomas are sporadically found inside the vertebral canal, compressing the spinal cord (4, 9). In these cases, compressive granulomas may result in severe spinal cord syndromes (4, 9); however, postvaccinal granulomas per se may result in significant economic losses for beef cattle industry due to costs involving the removal of affected parts in slaughterhouses (6, 7). To reduce postvaccinal reactions, the Brazilian Government decreased the recommended dosage from 5ml to 2ml and removed saponins from the formulation, although the vaccine is still based on mineral oil emulsion. Even though the vaccination status of the animals from this report is unknown, cattle raised in Mato Grosso (MT) should be vaccinated twice a year against FMD, according to the Brazilian National Program for Prevention and Eradication of Foot-and-Mouth Disease. In 2021, vaccination against FMD occurred in May, 60 days before cattle of this report were slaughtered; therefore, the lesions developed similarly to what was previously observed (4).

On gross evaluation, the multiple pyogranulomas closely resembled those seen in cases of systemic tuberculosis (3); however, the absence of central caseous necrosis and intralesional acid-fast bacilli and typical lesions in organs commonly associated with the mycobacterial infection, such as lung and lymph nodes, allow us to rule out this possibility (3). During microscopic examination, intralesional microorganisms were ruled out based on thorough examination of routine (hematoxylin & eosin) and histochemical stains (Grocott-Gomori's Methanamine Silver, Periodic acid-Schiff, Goodpasture, and Ziehl-Neelsen). In the absence of intralesional microorganisms, the clear vacuoles located within most pyogranulomas should not be interpreted as artifacts, considering that the clear spaces were well delimited and surrounded by an exuberant chronic inflammatory infiltrate, which is characteristic of reaction to oily agents (4, 9).

The intoxication by hairy vetch (*Vicia* spp.) or citric pulp and the consumption of feed containing diuredo-isobutane or silage containing the chemical conservant Sylade have been related to granulomatous diseases in cattle. However, the granulomatous reactions occurring in these intoxications tend to be systemic, while post-vaccinal vaccination is focal or focally extensive (1). None of these (tuberculosis or the intoxications described above) are associated with forming clear vacuoles centrally in the pyogranulomas. Other differentials must include causes of non-tuberculous chronic peritonitis/pleuritis. Chronic cases of traumatic reticuloperitonitis may affect extensive areas of the peritoneal cavity, with firm fibrous adhesions between gastrointestinal and sometimes diaphragmatic, pleural, or pericardial tissues (10). Definitive diagnosis is generally easily achieved through observation of intralesional

perforating foreign bodies. Here, there were no significant lesions in the reticulum (the most common injured site) in any of the cows. Additionally, the nodular appearance of the gross lesions and the absence of perforating bodies ultimately ruled out this possibility.

Mesothelioma is a common proliferative disorder among cattle's peritoneal and retroperitoneal neoplasms (8). Compared to the present case, this neoplasm may share some morphologic features due to its variable gross appearance and frequent spreading behavior into the coelomic cavity of origin (8). Besides, cavitory neoplasms are not expected to appear simultaneously in several animals, and the microscopic features of the present animals were consistent with a foreign body reaction. However, intralesional oil vacuoles do not exclude the existence of microbial agents, and you should always perform a complete diagnostic investigation.

Despite the atypical location of some pyogranulomas, the gross and microscopic lesions were highly compatible with other outbreaks involving postvaccinal reactions in beef cattle (4, 9), where animals from a same farm had identical lesions in the absence of intralesional microorganisms.

References:

1. Barros CSL, Figuera RA, Rozza DB, Rech RR, Sallis SV, Langohr IM. Doença granulomatosa sistêmica em bovinos no Rio Grande do Sul associada ao pastoreio de ervilhaca (*Vicia spp.*) Pesq Vet Bras 2001;21: 162-71.
2. Brasil. Ministério da Agricultura. Programa Nacional de Erradicação da Febre Aftosa: Plano estratégico 2017-2026 [Internet]. Brasília: MAPA, 2019 [cited 2022 Mar 15]. Available from: <http://www.agricultura.gov.br/febreaftosa>.
3. Caswell JL, Williams KJ. Respiratory system. In: Maxie MG, editor. Jubb, Kennedy & Palmer's Pathology of domestic animals. 6th Ed, Vol. 2. Philadelphia: Elsevier; 2016. p. 547-51.
4. Folchini NP, Zanatta L, Ugolini LW, Machado TP, Motta AC, Bohrer RR, Bondan C. Iatrogenic postvaccinal injection site granulomas in cattle. Acta Sci Vet 2018;46: 1-6.
5. Gaspar EB, Minho AP, Santos LR. Manual de Boas Práticas de Vacinação e Imunização de Bovinos. Embrapa Pecuária Sul; 2015 Aug. 10 p. Report No.: ISSN 1983-0475, Circular Técnica 47.
6. Leal PV, Pupin RC, Santos AC, Faccin TC, Surdi E, Leal CRB, Brumatti RC, Lemos AA. Estimativas de perdas econômicas causadas por reação granulomatosa local após uso de vacina oleosa contra febre aftosa em bovinos de Mato Grosso do Sul. Pesq Vet Bras 2014;34: 738-42.
7. Lima DCP, Costa AS, Ferreira MDS, Sobrinho JMF. Febre Aftosa: ocorrência de nódulo pós-vacinal segundo via de aplicação da vacina. Pesq Agropec 2014;20: 167-72.
8. Munday JS, Löhr CV, Kiupel M. Tumors of the alimentary tract. In: Meuten DJ. Tumors in Domestic Animals. 5th Ed. New Jersey: Wiley-Blackwell; 2017. p. 592-5.
9. Melo FG, Godoy ME, Facury Filho EJ, Carvalho AU, Meneses RM, Pierezan F. An outbreak of compressive myelopathy due to pyogranulomatous reaction to the oily adjuvant of the foot-and-mouth disease vaccine - case report. Arq Bras Med Vet Zootec 2019;71: 1453-8.
10. Uzal FA, Plattner BL, Hostetter JM. Alimentary system. In: Maxie MG, editor. Jubb, Kennedy and Palmer's Pathology of domestic animals. 6th Ed, Vol. 2. Philadelphia: Elsevier; 2016. p. 38-9.