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Case Report

A case of pneumonia and *Haemorrhagic gastroenteritis* in a six-year-old male Arewa horse

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ABSTRACT: Bacterial infections of the lower respiratory tract in adult horses occur when bacteria from the nasopharynx reach the lower airways and overwhelm normal defense mechanisms. Although *Streptococcus equi* subsp *zooepidemicus* is the most commonly isolated bacterial species, mixed infections are possible. Early intervention and appropriate antimicrobial selection result in a good prognosis for survival and return to athletic function in most horses. This case report described the laboratory diagnosis of pneumonia in a horse caused by bacterial organisms and haemorrhagic gastroenteritis. The case was diagnosed based on clinical signs, necropsy and bacteriological evaluation of tissue. The helminthology identification revealed *Gasterophilus* spp from the stomach. The bacterial isolates were identified through a combination of macroscopic examination of colony morphology, microscopic analysis using Gram staining, and confirmation with biochemical tests. Antimicrobial susceptibility profiles of the bacterial isolates were determined using disc diffusion methods. The major isolates identified were *Escherichia coli* and *Klebseilla spp*. Susceptibility of the bacteria to antibacterial agents showed the following profiles, resistance to ciprofloxacin, gentamicin, nitrofuration, ofloxacin, cefuroxime, ceftazidime, augmentin and co-trimoxazole. This case report described the importance of accurate laboratory diagnosis in achieving efficient treatment and reducing losses associated with an increased cost of treatment while highlighting the need for prudent antimicrobial use to combat the growing threat of antimicrobial resistance.

Keywords: Antimicrobial resistance, *Escherichia coli*, horse, *Klebsiella spp.*

INTRODUCTION

Respiratory diseases are the most common cause of illness and death in foals up to 12 months of age (Galvin and Corley, 2010) and are the fourth or fifth most common cause of death in the general horse population (Baker and Ellis, 1981; Pierezan *et al.*, 2009). Horses are frequently affected by bacterial pneumonia especially pneumonia due to sepsis. On the other hand, aged horses can develop primary pneumonia, which is often caused by *Streptococcus* *equi* subsp. *zooepidemicus* or *Rhodococcus equi* (Cohen, 2014; Reuss and Cohen, 2015). Horses may also develop bronchointerstitial pneumonia caused by equine influenza virus (Patterson-Kane et al., 2008). Pleuropneumonia and bronchopneumonia caused by *S. equi* subsp. zooepidemicus, are the most common diseases of the lower respiratory tract in adult horses (Carvallo *et al.*, 2017) and are often linked to stressful events (Oikawa *et al.*, 1994; Carvallo *et al.*, 2017). Pneumonia secondary to other disease processes, including oesophageal lesions, grass sickness or other forms of dysphagia, also frequently affects adult horses (Mair and Lane, 1989). Most inflammatory conditions of the small and large intestines in horses are of infectious origin, although there are a few non-infectious inflammatory conditions of importance. A significant number of severe inflammatory lesions in the small or large intestine remain of undetermined cause affecting horses of all ages. This disease is clinically characterized by diarrhea of not more than 10 days' duration, fever, anorexia, depression, and leucopenia; *Clostridium sordellii* (Songer and Uzal, 2005), *Actinobacillus equuli*, *Streptococcus equi*, *Histoplasma spp*., *Listeria monocytogenes*, and *Klebsiella pneumoniae* (Jang and Hirsh, 2002), among others, have been occasionally linked to enteritis and/or colitis in horses. Similarly, helminth infections, such as those caused by *Gasterophilus spp*., have also been associated with gastrointestinal disturbances. However, definitive evidence supporting their direct role in enteric disease remains limited (Weese and Brazier, 2005; Brown and Baker, 2007). Pneumonia and hemorrhagic gastritis in horses lead to significant economic burdens due to the costs of veterinary care, reduced performance, and potential loss of life (Uzal and Diab, 2015). Pneumonia, particularly from bacterial infections, can require costly long-term treatment and management, affecting racehorses and performance horses most notably due to extended recovery periods (McAuliffe *et al*., 2013). Hemorrhagic gastritis, often related to stress or non-steroidal anti-inflammatory drug (NSAID) use, can lead to high treatment expenses and can impair nutrient absorption, impacting horse condition and performance (Murray *et al*., 1989). This case report highlights the critical importance of accurate diagnosis and the appropriate selection of medications in the effective treatment of equine diseases. Timely and precise identification of the underlying condition not only improves treatment outcomes but also minimizes complications and reduces the risk of drug resistance.

CASE REPORT

History

A six-year-old male Arewa horse was referred to the Large Animal Clinic, Federal College of Animal Health Clinic, Vom, sick and recumbent, tentative diagnosis of tetanus with inadequate history, which lasted for two days. A nail was removed from the left forelimb 3 weeks ago. The animal was fed with hay and bran (wheat offal). The drugs administered were Imidocarb dipropionate, Benzyl penicillin, Intravenous fluid, xylazine, Diclofenac and multivitamin. It was vaccinated 3 weeks prior to presentation with tetanus toxoid.

Clinical signs

The clinical signs presented are sweating, high temperature (41°C), congested ocular mucous membrane, dilation of the nostrils, restlessness, recumbency and generalised edema around the head and neck.

Postmortem

The heart was slightly enlarged, and the right ventricle was filled with chicken fat. The lungs were congested, haemorrhagic and heparized. There were frothy exudates in the trachea. There was severe haemorrhagic gastroenteritis, the stomach colon were filled with worms. The liver was enlarged, the kidneys were congested, and there was nephritis.

Tentative diagnosis

The tentative diagnosis for the horse was Pneumonia, Hemorrhagic gastroenteritis, and Helminthosis, indicating concurrent respiratory infection, severe intestinal inflammation with bleeding, and parasitic infestation.

Sample collected

A lung sample was collected to perform a culture for isolation of the microorganism, followed by identification to determine the specific microorganism responsible for the condition, and a susceptibility test to assess the effectiveness of various antimicrobial agents in guiding targeted treatment. A helminth sample from the stomach was sent to the helminthology laboratory.

Helminthology examination

The identification of the parasite was conducted by the parasitology division of the National Veterinary Research Institute, Vom, using standard diagnostic techniques.

Microbiological examination

In the laboratory, the lung sample was smeared with a sterilized spatula and a loop was used to culture on blood on MacConkey Agar. Then it was incubated at 37°C for 24 hours. The positive samples were then subcultured to obtain a pure colony.

Catalase test

A sterilised loop was used from the colony on blood agar and placed on a clean glass slide and a drop of hydrogen peroxide was dropped on the colony and there was bubble formation.

Biochemical test

Isolates presumptive of *Escherischia coli* and *Klebsiella* spp were inoculated into Tripple Sugar Iron (TSI), Indole, Citrate, Urea, Methyl Red, Voges Proskauer and motility and was incubated for 24 hours at 37°C. Each test was read by the colour change observed.

Sugar test

The presumptive isolate of *Klebsiella* spp was subjected to a sugar fermentation test which included Lactose, Trehilose, Fructose, Ornithine I, Ornithine II, Sucrose, Arabinose, Glucose, Mannitol, Rafinnose, Maltose, Xylose and was incubated for 24 hours at 37°C. The result was read by the colour change observed and in comparison, to the control.

RESULTS

Parasitology

The helminth was confirmed to be *Gasterophilus* spp. This genus, commonly referred to as bot flies, is identified based on morphological characteristics of the larval stages, such as body segmentation and spines. This identification helps inform treatment strategies for managing parasitic infestations in affected animals, as *Gasterophilus* larvae are known to impact the gastrointestinal tract (Plate 1). With the morphologic characteristics of the larvae as robust and cylindrical with spiny bands on each segment; distinct oral hooks for attachment to the host's tissues

Plate 1. Larvae of *Gasterophilus* spp showing robust and cylindrical with spiny bands in each segment in the stomach of the horse.

Microbiological

On blood Agar, a small, raised, smooth, moist, whitish, non-haemolytic colonies upon Gram stain showed short rod gram negative which is presumptive for the identification of *Corynebacterium* spp (Plate 2c). On MacConkey, two colonies were seen, one was a medium, raised, smooth, moist, pinkish lactose fermenter and the other was a tiny, raised, smooth pinkish, non-lactose fermenter. Each of these colonies was subcultured. On Gram stain, the latter was seen as short rods Gram-positive rods which was presumptive identification for *Klebsiella* spp and the other was seen as long rods Gram-negative which was suggestive of *Escherichia coli*.

After inoculation and incubation for 24 hours at 37°C on Eosin methylene blue agar (EMB), MacConkey agar, and Brilliant green agar as selective media, the growth and differentiation of microorganisms can be observed based on colony colour and morphology, which helps in identifying lactose fermenters, gram-negative bacteria, and distinguishing between different types of pathogens. A distinctive metallic green sheen was observed which was due to the fermentation of lactose which lowers the pH and causes the dyes in the agar (EMB) to precipitate. This is presumptive of *E. coli* (Plate 2a). Following incubation, there was formation of a large, mucoid, and pink colonies due to lactose fermentation was seen. Lactose fermentation leads to acid production, turning the medium pink around colonies was observed on MacConkey agar which is presumed to be *Klebsiella spp* (Plate 2b). Microscopic examination after gram staining of both organisms shows that *Escherichia coli* gram-negative rod (bacillus) appears pink under the microscope due to the thin peptidoglycan layer that does not retain the crystal violet-iodine complex after decolourization. While *Klebsiella pneumoniae* was also a gram-negative rod, appears pink with prominent capsules visible as a halo under appropriate staining (Plate 3a and 3b). *E. coli* was further subjected to biochemical tests which showed lactose fermentation-positive, indole test: negative for *Klebsiella pneumoniae*, urease: positive, citrate utilization: positive, triple sugar ion: acid/acid with gas, methylred test: negative, Voges-proskauer (VP) test: positive and motility: negative (non-motile) (Plate 4). While for *E. coli,* the result revealed indole test: positive, methyl red (MR) test: positive, Voges-proskauer (VP) test: negative, triple sugar

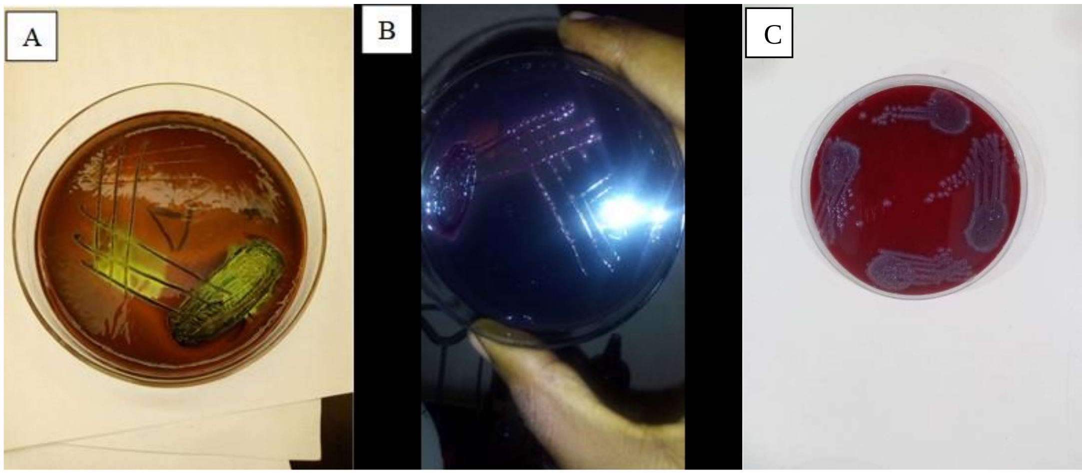

Plate 2. Culture on the plates: **(a).** *E. coli* on Eosin Methylene Blue; **(b).** *Klebsiella spp* on MacConkey; **(c)** *Corynbacterium* on blood agar.

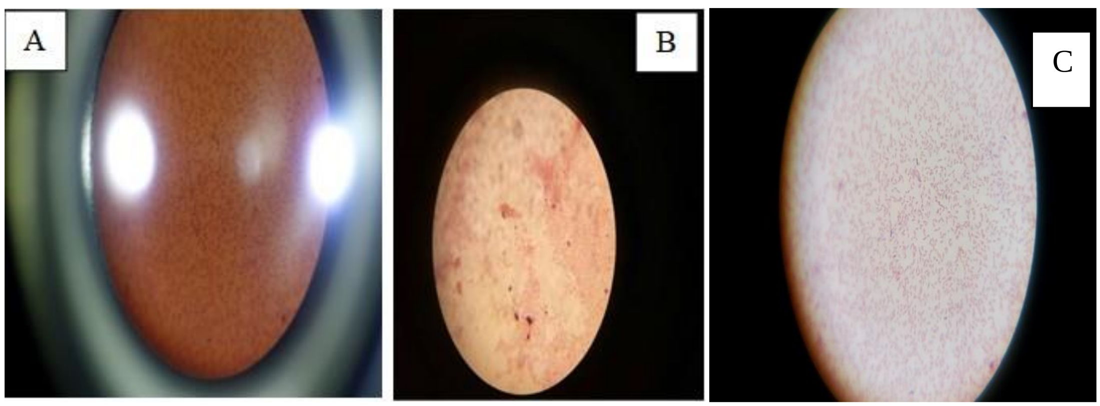

Plate 3. Microscopic identification: **(a)** *Klebsiella spp;* (b). *E. coli c) Corynbacterium* at X 40magnification.

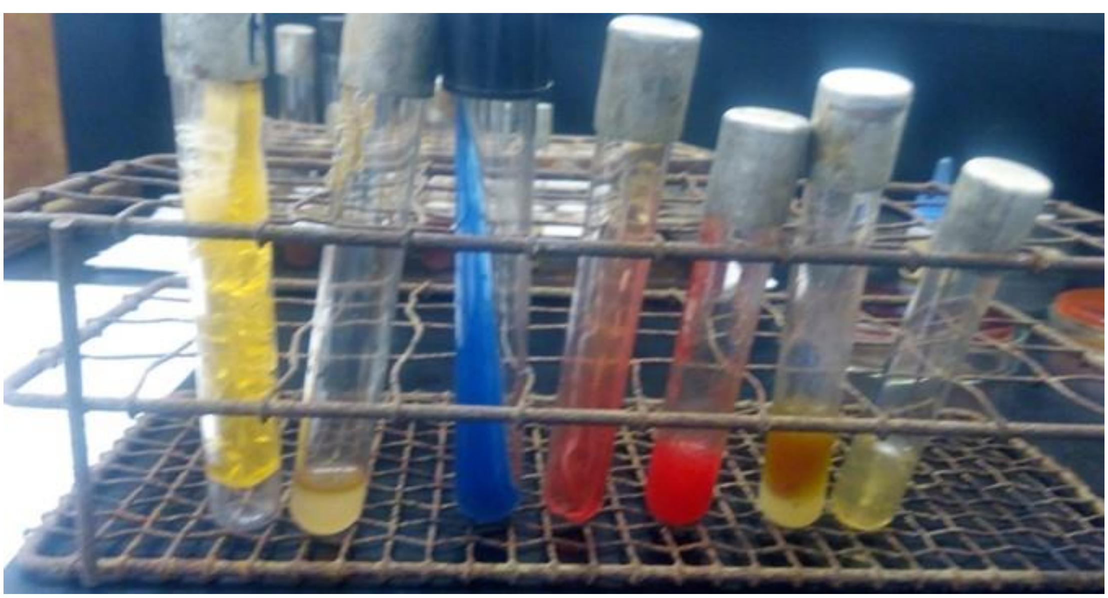

Plate 4. Biochemical test: *Klebsiella* spp.

Plate 5. Biochemical test: *E. coli.*

Table 1. Biochemical test of *Escherischia coli* and *Klebsiella* spp for identification

Biochemical test	Lactose fermenter	Non lactose fermenter
TSI	Acid/acid + gas	Acid/acid + gas
Indole	Positive	Negative
Citrate	Negative	Positive
Urea	Negative	Positive
Methyl red	Positive	Positive
Voges Proskeuar	Negative	Negative
Motility	Motile	Motile
	Escherischia coli	*Klebsiella spp*

ion: acid/acid with gas, citrate utilization: negative, urease test: negative, catalase test: positive, oxidase test: negative, glucose fermentation: positive (with acid production) (Plate 5). Table 1 shows the result clearly. A sugar fermentation test for *Klebsiella pneumoniae* reveals its ability to ferment a variety of sugars, often producing acid and gas. The test was done with a concurrent control to compare and standardize the test. Results reveal positive for glucose (acid and gas production), positive for lactose (distinguishes it from non-lactose fermenters like *Salmonella*), positive for sucrose, and negative for maltose and mannitol in some strains (Plates 6 and 7) as shown in Table 2.

Table 2. Sugar fermentation test for *Klebsiella pnuemoniae.*

Sugars	Result
Lactose	Positive
Trehilose	Negative
Fructose	Negative
Ornithine 1	Negative
Ornithine 2	Negative
Sucrose	Positive
Arabinose	Negative
Glucose	Positive
Mannitol	Negative
Raffinose	Positive
Maltose	Negative
Xylose	Negative

Antibiotic susceptibility

The result showed resistance of E. coli and Klebsiella spp. to multiple antibiotics, including ciprofloxacin, gentamicin, nitrofurantoin, ofloxacin, cefuroxime, ceftazidime, augmentin, and co-trimoxazole, reflects a significant challenge in treating infections caused by these pathogens. The resistance could be due to various mechanisms, such as the production of β-lactamases (including ESBLs), mutations

Plate 6. Sugar fementation test (control).

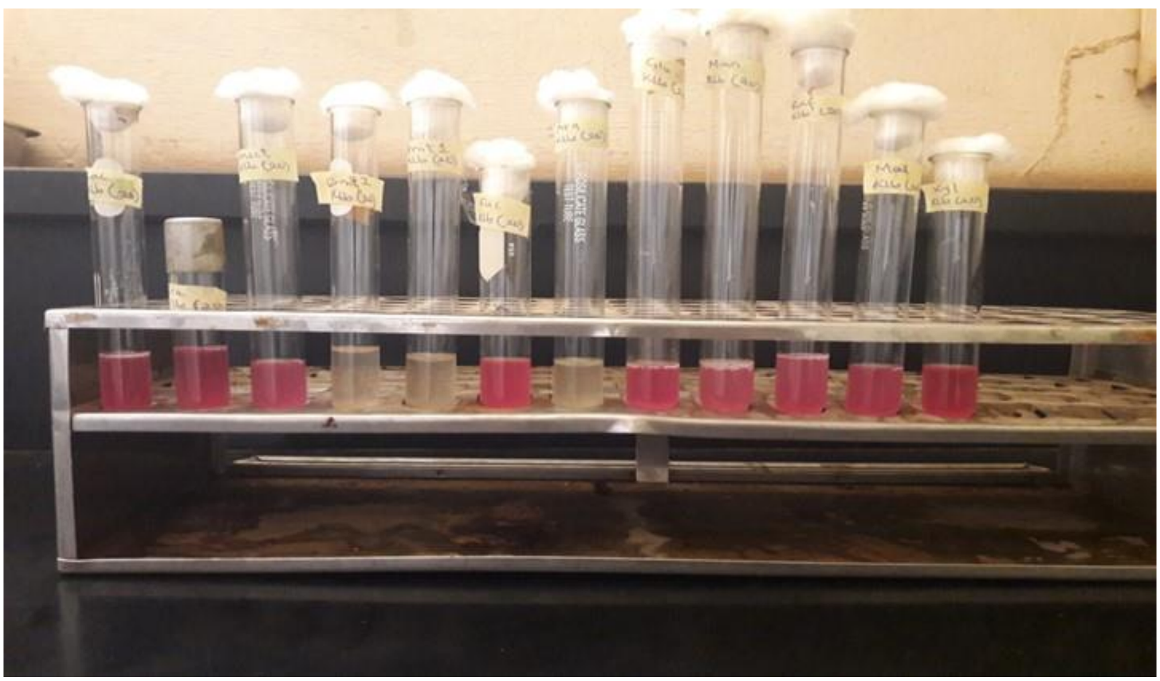

Plate 7. Sugar fementation test (test): *Klebsiella pnuemoniae*.

in drug target sites, reduced drug uptake, or increased efflux. This antibiotic resistance highlights the importance of antimicrobial stewardship, surveillance, and the need for alternative treatment options in managing infections caused by resistant strains of *E. coli* and *Klebsiella spp* (Plate 8a and b).

DISCUSSION

In the past decade, there have been significant strides in the diagnosis, treatment, and prevention of many conditions affecting the upper and lower airways of the horse (Kozłowska *et al*., 2022). Vast advances in technology

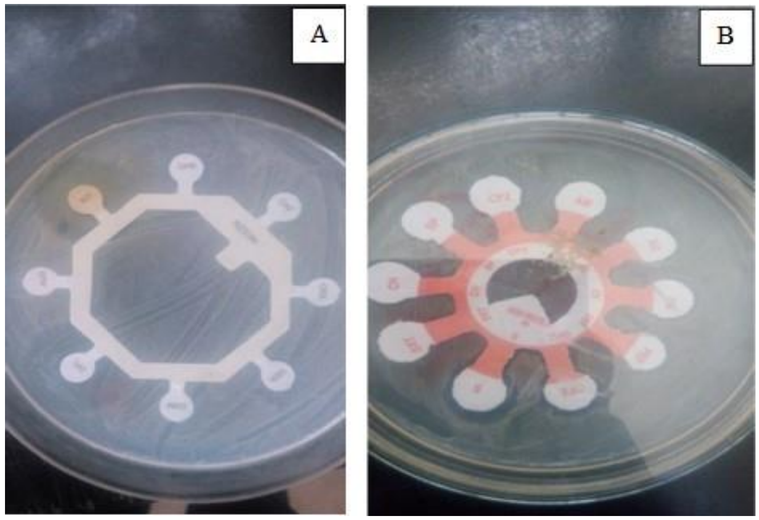

Plate7. Antimicrobial susceptibility test for the (a) *E. coli*, (b) *Klebseilla spp* and using the disc diffusion method with zones of inhibition.

have allowed for improved diagnostic imaging with the routine use of digital radiography and ultrasound as well as increased availability of dynamic endoscopy, computed tomography, and MRI (Reuss and Chesen, 2015). Researchers have furthered our knowledge of the etiologic organisms causing many of the infectious diseases of the respiratory tract, thus allowing for improved preventative strategies. They have also further elucidated the pathophysiology of frustrating diseases such as recurrent airway obstruction, inflammatory airway disease, and exercise-induced pulmonary haemorrhage. New antimicrobials have been discovered and validated for use in equines. Despite these many advances, the respiratory system continues to be a challenge for veterinarians and horse owners (Reuss and Giguère, 2015).

In these cases, the major bacteria organisms isolated were *Escherichia coli* and *Klebseilla* spp. The helminth identified was *Gasterophilus spp*. This agreed with the report of Estell *et al*. (2015) where *Klebsiella* spp. infection was considered in horses presenting with signs of hemorrhagic pneumonia because 15/46 horses presented with hemorrhagic nasal discharge. Although nasal discharge may have represented epistaxis because of coagulopathy, discharge often was reported to be dark and increased after coughing, indicating that it may have originated from the lower respiratory tract. One striking finding of this case report was the accurate diagnosis of a case of *Gasterophilus* spp from the stomach of the horse. This may be a result of a poor deworming regimen and management practice of the horse. This also follows the study of Pawlas-Opiela *et al*. (2022) stating that *G. intestinalis* infections, seem to be a sensible completion of the diagnostic and decision-making process concerning deworming the herd. Furthermore, the isolation of the *Gasterophilus* spp from the stomach could be attributed to the weakened immunity of the horse that is burdened with bacterial infection and poor management practice. The most prevalent form of gastritis is associated with ulceration of the pars esophagea. Difficulties still exist in establishing the causes of a significant number of enteric diseases in these horses. This problem is compounded by several agents of enteric disease also being found in the intestines of clinically normal horses, which questions the validity of the mere detection of these agents in the intestine or stomach (Uzal and Diab, 2015).

This case report also observed the antimicrobial resistance of these bacterial organisms to ciprofloxacin, gentamicin, nitrofurantoin, ofloxacin, cefuroxime, ceftazidime, augmentin, and co-trimoxazole indicating a persistent raise into antimicrobial resistance. This report agrees with a study conducted by Estell *et al*. (2015) where they also recorded multi-drug resistance from respiratory bacteria isolated from horses. This is a potential threat to the equine industry.

Conclusions

This case report described the importance of accurate laboratory diagnosis in achieving efficient treatment and reducing losses associated with increased cost of treatment. Also, it emphasized the rise in antimicrobial resistance in animals. Furthermore, the isolation of *E. coli, Klebsiella* and *Corynebacterium*, from the lungs of the horses indicates potential contamination issues within the production and storage chain. The identification of *Gasterophilus* spp in the stomach of this horse indicates poor nutritional condition of the horse and hygiene as well. Ensuring strict environmental and hygienic conditions, good nutritional conditions and adherence to good management practices in equine production can mitigate these risks and enhance the safety of horses.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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REFERENCES

Baker, J. R., & Ellis, C. E. (1981). A survey of post mortem findings in 480 horses 1958 to 1980:(1) causes of death. *Equine Veterinary Journal*, *13*(1), 43-46.

Brown, C. C., & Baker, D. C. (2007). Infectious diseases of the gastrointestinal tract. In: *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, Sixth Edition*, Volume 2, Science Direct.

Carvallo, F. R., Uzal, F. A., Diab, S. S., Hill, A. E., & Arthur, R. M. (2017). Retrospective study of fatal pneumonia in racehorses. *Journal of Veterinary Diagnostic Investigation*, *29*(4), 450-456.

Cohen, N. D. (2014). Rhodococcus equi foal pneumonia. *Veterinary Clinics: Equine Practice*, *30*(3), 609-622.

Estell, K. E., Young, A., Kozikowski, T., Swain, E. A., Byrne, B. A., Reilly, C. M., Kass, P. H., & Aleman, M. (2016). Pneumonia Caused by K lebsiella spp. in 46 Horses. *Journal of Veterinary Internal Medicine*, *30*(1), 314-321.

Galvin, N. P., & Corley, K. T. T. (2010). Causes of disease and death from birth to 12 months of age in the Thoroughbred horse in Ireland. *Irish Veterinary Journal*, *63*(1), 37-43.

Jang, S. S., & Hirsh, D. C. (2002). Characterization of Pasteurella multocida and Klebsiella pneumoniae isolated from horses. *Journal of Veterinary Diagnostic Investigation*, *14*(4), 396-399.

Mair, T. S., & Lane, J. G. (1989). Pneumonia, lung abscesses and pleuritis in adult horses: a review of 51 cases. *Equine Veterinary Journal*, *21*(3), 175-180.

McAuliffe, S., Sanchez, L. C., & Giguère, S. (2013). Pharmacokinetics of antimicrobials and implications for treatment of bacterial pneumonia in foals. *Veterinary Clinics of North America: Equine Practice,* 29(1), 71-84.

Murray, M. J., Grodinsky, C., Anderson, C. W., Radue, P. F., & Schmidt, G. R. (1989). Gastric ulcers in horses: A comparison of endoscopic findings in horses with and without clinical signs. *Equine Veterinary Journal, 21*(S7), 68-72.

Oikawa, M., Takagi, S., Anzai, R., Yoshikawa, H., & Yoshikawa, T. (1995). Pathology of equine respiratory disease occurring in association with transport. *Journal of comparative pathology*, *113*(1), 29-43.

Patterson-Kane, J. C., Carrick, J. B., Axon, J. E., Wilkie, I., & Begg, A. P. (2008). The pathology of bronchointerstitial pneumonia in young foals associated with the first outbreak of equine influenza in Australia. *Equine veterinary journal*, *40*(3), 199-203.

Pawlas-Opiela, M., Jawor, P., Galli, J., Zak-Bochenek, A., Gorczykowski, M., Galli, J., Sołtysiak, Z., & Stefaniak, T. (2022). The relationship between the intensity of *Gasterophilus intestinalis* larvae infection and the serum and salivary humoral immune response in horses. *Scientific Reports*, *12,* Article number 17573.

Pierezan, F., Rissi, D. R., Rech, R. R., Fighera, R. A., Brum, J. S., & Barros, C. S. (2009). Necropsy findings related to the cause of death in 335 horses: 1968-2007. *Pesquisa Veterinária Brasileira*, *29*, 275-280.

Reuss, S. M., & Chesen, A. B. (2015). Updates on respiratory medicine and surgery. *Vet Clin Equine* 31, 15-16.

Reuss, S. M., & Cohen, N. D. (2015). Update on bacterial pneumonia in the foal and weanling. *Veterinary Clinics: Equine Practice*, *31*(1), 121-135.

Reuss, S. M., & Giguère, S. (2015). Update on bacterial pneumonia and pleuropneumonia in the adult horse. *Veterinary Clinics: Equine Practice*, *31*(1), 105-120.

Songer, J. G., & Uzal, F. A. (2005). Clostridial enteric infections in pigs, horses and calves*. Journal of Veterinary Diagnostic Investigation*, *17*(6), 528-536.

Uzal, F. A., & Diab, S. S. (2015). Gastritis, enteritis, and colitis in horses. *Veterinary Clinics: Equine Practice*, *31*(2), 337-358.

Weese, J. S., & Brazier, J. S. (2005). *Equine clinical microbiology.* Elsevier Health Sciences.