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Mixed Infection by *Anaplasma marginale* and *Anaplasma centrale* in Buffalo: A Short Follow-Up of a Case

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ABSTRACT

The buffaloes with mixed infections of *Anaplasma marginale* and *A. centrale* may cause severe clinical manifestation and difficulty in treatment, particularly in the field setting when diagnostic facilities are limited. **Objectives:** To report about a case of mixed *Anaplasma* infection in a buffalo heifer, explain clinical manifestations, laboratory diagnosis, response to treatment, and emphasize on the significance of evidence-based management. **Methods:** A buffalo heifer with clinical presentation of fever, emaciation, weight loss, shortness of breath, cough, anemia, fatigue, isolation from the herd, and anorexia was studied. Giemsa-stained thin blood smears were used to conduct laboratory diagnosis to confirm the presence of *A. marginale* and *A. centrale*. Fecal sample was examined for metazoan parasites. The animal was given three normal doses of oxytetracycline with antipyretics and multivitamins separated by an interval of 24 hours. It was also observed that prior empirical treatments were done by local veterinary technicians. **Results:** Laboratory analysis proved the presence of both *A. marginale* and *A. centrale*. Fecal analysis showed negative metazoan parasite. The buffalo did not respond to the oxytetracycline treatment that was administered to it, and antipyretics made its symptoms relieve only for a short period. Earlier treatments that were not validated in a laboratory were not effective. **Conclusions:** The case in question highlights the need to test anaplasmosis unambiguously in the laboratory in terms of appropriate diagnosis and therapeutic management. It also points out the issues which are related to lack of standardization of treatment practices and poor diagnostic facilities in the field setting.

INTRODUCTION

Anaplasmosis (Gall-sickness) is a domestic/wild ruminant rickettsial infection of red blood cells which is transmitted by vectors [1, 2]. It is the disease caused by six species of the genus *Anaplasma* such as *A. marginale*, *A. centrale*, *A. ovis*, *A. platys*, *A. phagocytophilum*, and *A. bovis* [3]. The most important of these are *A. marginale* and *A. centrale* that have been known to cause different levels of hemolytic anaemia, fever, emaciation and low productivity in bovines. An anaplasmosis, which is more severe, is caused by *A. marginale* and a milder infection is caused by *A. centrale* [4, 5]. It is primarily transmitted by ticks belonging to the genera *Boophilus*, *Dermacentor* and *Rhipicephalus*, but can

also be spread by biting flies or mechanically with contaminated equipment (e.g. needles, dehorning or tagging tools) [6, 7]. Dual infections of two species of *Anaplasma* are extremely infrequent, but can complicate case diagnosis and treatment care [8, 9]. The disease occurs in four phases, including incubation, developmental, convalescent, and carrier, the incubation period takes 3-9 weeks. Acute anaemia and clinical manifestation of the disease occur in the developmental phase, and finally it cures but with the carrier status lifelong [10, 11]. The diagnosis of bovine anaplasmosis in Pakistan rests on clinical manifestations rather than on



confirmatory laboratory tests thereby causing misdiagnosis and wrong treatment [12, 13]. The current case report of a rare mixed infection of *A. marginale* and *A. centrale* in a buffalo heifer, which was confirmed by the examination of the Giemsa-stained blood smears and highlights the importance of correct diagnosis and evidence-based treatment in the field.

This study aimed describe a case of mixed *Anaplasma* infection in a buffalo heifer, report on clinical presentation, laboratory diagnosis, response to treatment and emphasize the role of evidence-based management.

METHODS

It was a case study of clinical case report. A buffalo heifer 2.5–3 years old with apparent clinical features indicative of anaplasmosis was observed around the University of Peshawar, Pakistan, between 18 August 2017 and 28 August 2017. The ethical approval was taken from Institutional Review Board with IRB no: 10719. As per the description of the owner, the animal was bought on 07/08/2017 in the local livestock market as a business animal. Clinical symptoms have manifested themselves at the time of purchase, and the owner confused them with temporary effects of transport stress and extreme summer heat. Monitoring of the case involved a 10 days' follow-up on the case after initial identification and after which the animal was sold. The rectal temperature measures were taken daily, and mucous membranes of the vagina and eyes were observed on the presence of anemia. A fecal sample on 18/08/2017 was tested through simple Lugol Iodine stained smear of helminth ova. In case of blood examination, the ear vein was pricked with a sterile needle, and it was stained with freshly prepared Giemsa working solution. A second blood smear was prepared by using erythrocytes that were subjected to the Ficoll density gradient centrifugation technique that reduced contamination of leukocytes and debris. Thirdly, Oxytetracycline (24-hour intervals) was administered in combination with antipyretics and multivitamins in three standard doses to the animal. The surface of the whole body was thoroughly checked on the presence of ticks which are the main vectors of anaplasmosis but the presence of ticks was not found in the clinical examination. In the treatment process, high levels of uncertainty in veterinary practice were experienced. The owner initially visited a local dispenser 09/08/2017, which was two days after the purchase and was provided with unspecified injectable medications, details of which were not disclosed to the owner. This first dispenser was not successful, and another dispenser was consulted who prescribed one injection of an unspecified antibiotic and an antipyretic. Subsequently, the case was assumed by the study that did a regular healing cycle of oxytetracycline and supportive antipyretics after the diagnosis was established. The study workers were only able to administer three doses with

intervals of 24 hours and the owner withdrew the treatment. This was due to cessation because he was advised by another veterinarian who used Imdocarb, an antiprotozoal medication. This tendency to alternate between unqualified dispensers and veterinarians is a typical and alarming tendency among livestock owners, which, in most cases, is determined by desperation and ignorance, a drowning man is holding on to a straw. Illiterate and poor peasants and farmers are still very susceptible to exploitation by the quacks and the veterinary practitioners who have limited experience. It is also aggravated by the lack of well-equipped husbandry healthcare facilities and veterinary hospitals, which emphasizes the necessity of more powerful veterinary outreach and community education.

RESULTS

The animal presented persistent severe symptoms including fever, emaciation and weight loss, short breath, cough, anaemia, fatigue, isolation from the herd, frequent sitting in water, and anorexia. Anaemia (A), short breath (B), weight loss (C), and fluctuations in body temperature during 10-day follow-up (D). Over the 10-day follow-up period, the body temperature fluctuated between 102°F and 104°F. The animal was in the acute developmental stage of infection. It did not respond to the standard three-dose treatment regimen of oxytetracycline. However, administration of antipyretics provided temporary relief, easing shortness of breath and apparent discomfort, which might be attributed to a transient reduction in body temperature and improved oxygen solubility in blood (Figure 1).



Figure 1: Clinical Symptoms of Anaplasmosis in Buffalo

A Giemsa-stained thin blood smear examination revealed a mixed infection with *A. marginale* and *A. centrale*, while the stool smear was found negative for helminth ova. (A) Vials of medication are administered by the local dispenser. (B) *A. marginale* (white arrows) and *A. centrale* (blue arrows) observed in Giemsa-stained thin smear at 1000× magnification (oil immersion). (C) Vacuolar artefacts in stool smear (orange arrows). Scale bar = 10 µm. The Giemsa-stained smear prepared from density gradient-isolated red blood cells (RBCs) gave a clearer and more

distinct picture as compared to the direct smear obtained from venepuncture. The latter sample exhibited greater contamination with skin debris, even after thorough cleansing of the area with an alcohol swab before sampling. No external tick infestation was observed during examination (Figure 2).

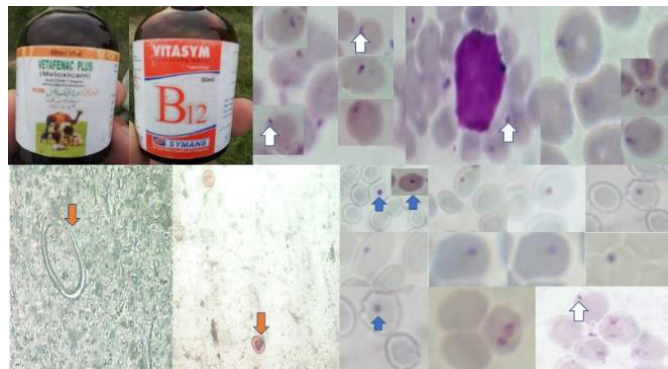


Figure 2: Microscopic and Treatment Observations

DISCUSSION

Microscopic observation in Romanowsky-or Giemsa-stained thin blood or tissue smears is one of the several diagnostic techniques used for *Anaplasma* species [14, 15]. It is the oldest and most conventional diagnostic method, and requires considerable technical expertise and experience on the part of laboratory technicians [16, 17]. It has been reported that with only 10^6 /mL RBC infectivity, the diagnostic sensitivity becomes markedly limited. Despite these limitations, it remains the most widely adopted tool for diagnosing anaplasmosis, especially effective in identifying the acute phase of infection [18, 19]. Viewing the results of our study, separation of the RBCs by the density gradient centrifugation technique is an effective method to eliminate leukocytes and creates a smear which is more homogenous and artefact-free. The detection of intraerythrocytic *Anaplasma* species is much more evident with only erythrocytes observable by the microscope. Elution of the impurities and precipitates of the supernatant also reduce the possibility of artefactual misinterpretation. These smears are consequently of special use in the identification of intraerythrocytic *Anaplasma* species. The age of animals infected had a high correlation with the severity of clinical symptoms. More severe in cattle aged 1-3 years and the best morbidity in cattle aged above 3 years with an estimated rate of mortality of 30-50%. This age-symptom correlation is of special importance to our study. The heifer had serious clinical manifestations, which were in line with past observations [20]. The current case has shown that acute anaplasmosis was not responsive to common treatment programs with oxytetracycline and imidocarb, just as it was observed in previous research [21]. Such non-response can be explained by some potential causes, such as

antimicrobial resistance, a highly developed infection, or a mixed infection of many *Anaplasma* species. The ignorance of livestock owners and the limited regulation are some of the factors that have enhanced the growth of unqualified and inexperienced veterinary practitioners, especially in the rural communities. Though veterinary ethics and animal welfare principles are included in professional training, they tend to be less than optimal when they are followed in the field. Other veterinarians can make their treatment choices based on presumptions of the economic limitations of their owner by choosing less expensive or less thorough treatment courses of action that can affect both the effectiveness of treatment and the welfare of the animal [22]. In the current case, it was noted that the animal had first been dispensed by unqualified veterinary dispensers who failed to explain to the owner what type of therapy, the dosage, and the use of the therapy was, and who was a poor and illiterate peasant.

CONCLUSIONS

The present study warns of impure veterinary healthcare arrangements in Khyber Pakhtunkhwa and Pakistan in general. Farmers' awareness regarding veterinary health medicine is extremely poor, due to which they become easy prey to quack and inexperienced veterinarians and veterinary technicians. Laboratory diagnosis of veterinary diseases is extremely poor due to a lack of diagnostic facilities (only the Veterinary Research Institute (VRI) in the whole Peshawar district has the facility).

Authors Contribution

Conceptualization: QJ

Methodology: QJ, MUH, FA, FS, JS

Formal analysis: FS, JS

Writing review and editing: I, MU

All authors have read and agreed to the published version of the manuscript

Conflicts of Interest

All the authors declare no conflict of interest.

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