NIGERIAN VETERINARY JOURNAL

ISSN 0331-3026

Nig. Vet. J., March 2021 Vol 42 (1): 60 – 70.

https://dx.doi.org/10.4314/nvj.v42i1.4 **ORIGINAL ARTICLE**

Pathologic Changes in Lung Morphology in Experimental *Klebsiella pneumoniae* and *Trypanosoma brucei* co-Infection in West African Dwarf Goats

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SUMMARY

The bacteria *Klebsiella pneumoniae* easily takes advantage of compromised immune status to induce pathologies on their hosts. This study investigated pulmonary pathomorphologies associated with experimental co-infection of *Klebsiella pneumoniae* and *Trypanosoma brucei* in West African Dwarf goats. Fifteen clinically healthy male goats of about six months of age were used in this study. They were randomly assigned into five groups of three each. Group I was the uninfected/negative control; Group II was infected with *K. pneumoniae* only as positive control I; Group III with *T. brucei* followed by acute *K. pneumoniae* infection at day 7 post *T. brucei* infection; Group IV with *T. brucei* followed by sub-acute *K. pneumoniae* at day 14 post *T. brucei* and Group V with *T. brucei* only, as positive control II. Throughout the study period, *K. pneumoniae* was only re-isolated from goats in Grp IV. Grossly, no lesions were observed in the lungs of goats in Grp II, while lungs of goats in Grp III showed mild consolidation in the cardiac lobes. Lungs of Grp IV goats showed multifocal abscessation and red hepatisation, and Grp V had widespread hyperaemia in all lobes. The histology showed mild intra-alveolar exudations and alveolar collapse in Grp II, mild to moderate cellular infiltrations and sero-fibrinous exudations in Grp III, and severe bronchopneumonia with fibrino-purulent exudations in Grp IV. Grp V had generalised oedema and hyperaemia. This study has shown that *Trypanosoma brucei* immunosuppresses infected goats, making them vulnerable to pneumopathies from *Klebsiella pneumoniae.*

Key words: *Klebsiella pneumoniae, Trypanosoma brucei*, pneumopathies, immunosuppression

INTRODUCTION

The importance of the West African Dwarf (WAD) goat, especially to the rural economy of the West Africa sub-region has been well documented (Wilson, 1991; Chiejina and Behnke, 2011). Its importance has been attributed to a lot of factors including the excellent adaptation of this breed to its native habitat, its high fertility and its reproductive prolificacy (Chiejina and Behnke, 2011). Other very important attributes of the West African Dwarf goat includes their tolerance to trypanosomosis (Trypanotolerance) and to gastrointestinal haemonchosis (Haemonchotolerance) (Chiejina and Behnke, 2011). In fact, the predominantly small-scale

rural goat keepers in the West Africa sub-region successfully rear these animals without having to rely on trypanocides and anthelmintics - which by the way are neither cheap nor readily available to most of them (Chiejina and Behnke, 2011). In south-east Nigeria, they are traditionally reared, free-roaming in an extensive management system, and only confined/tethered during the farming season (Francis, 1988). This practice easily allows them to be exposed to various disease conditions.

Pneumonia is a common finding in small ruminants, but much of the literature on pneumonia in goats has centred on viral pneumonias and pneumonias from bacteria species other than Klebsiella. *Klebsiella* spp members of the gut bacteria (enterobacteriaceae), are ubiquitous and known to survive in soil and vegetation for very long periods due to their high survivability nature. The bacteria have been isolated from nasal cavities of man and are known to be shed in the faeces of cattle (Verbist*et al.,* 2011). There are several known species of the Klebsiella genus, all possessing similarities in DNA homology. However, *Klebsiella pneumoniae*, a gram-negative, non-motile, facultative anaerobe is the most clinically important member of the group. Like other members, the lipopolysaccharide (O antigen) and the capsular polysaccharide (K antigen) are largely responsible for the pathogenicity of this organism (Podschun and Ullman, 1998; Umeh, 2011). *Klebsiella pneumoniae* is a typically opportunistic bacterium which readily takes advantage of immune compromised individuals (Ningthoujam, 2011).

Trypanosomosis is a disease of considerable economic importance in the Tropics. Its study and control has become a priority everywhere in the African continent where development programmes for ruminant and other livestock management take place. The phenomenon of Trypanotolerance has been well established in Zebu, Ndama and Muturu breeds of cattle (Murray *et al.,* 1977), but this trait is not as well elucidated in the WAD goats as has been in their larger ruminant counterparts. Parasitaemia in trypanosomosis is usually associated with anaemia and leucopenia in affected animals with an attendant immunosuppression (Godwin *et al.,* 1972). The trypanotolerance in tolerant breeds like the WAD is a reference to reduced susceptibility and not to actual or total resistance (Godwin, 1972; Murray *et al.,* 1979a). That being the case, it was interesting to investigate the possible consequences of the reported "immunity" in this breed in the presence of a concurrent infection.

This study sought to assess if a field isolate of *Klebsiella pneumoniae* from cattle will be infective to WAD goat, and to compare the pulmonary lesions of single and concurrent *Klebsiella pneumoniae* and *Trypanosoma brucei* infections in West African Dwarf goats.

MATERIALS AND METHODS

Experimental animals

Fifteen (15) clinically healthy young male West African Dwarf goats of about 6 months of age were used for this study. The goats were allowed 2 weeks of acclimatization and their nares screened to ensure there were no prior Klebsiella infection. The goats were housed in well ventilated fly-proof pens and fed with browse plants and provided water *ad libitum*.

Experimental design and methods

The goats were randomly assigned into five (5) groups of three (3) goats each;

I. Group I – Uninfected, negative control.

II. Group II – *Klebsiella pneumoniae* positive control I.

III. Group III – *T. brucei* + *K. pneumoniae*, acute

IV. Group IV – *T. brucei* + *K. pneumoniae* sub-acute

V. Group V – *Trypanosoma brucei* positive control II

NB: Groups III and IV were infected with *K. pneumoniae* at days 7 and 14 respectively post-*T. brucei* infection,

All procedures in the study were in accordance with the prescribed guidelines of the Institutional Animal Ethics Committee.

***Trypanosoma brucei* inoculum**

The *T.brucei* used in this study was obtained from the Department of Veterinary Parasitology of the Faculty of Veterinary Medicine, Federal University of Agriculture, Makurdi, Benue state, North Central Nigeria. Blood containing 1.13 x 10^8 parasites/ml of blood from *Trypanosoma brucei*- infected donor goat was diluted with glucose saline at the ratio of 1:3. One ml of the resulting mixture was used to infect each goat in Groups III, IV and V by intravenous injection via the cephalic vein. Parasitaemia was detected in the blood by day 7 post-infection on wet mount by the direct microscopy method described by Herbert & Lumsden (1976).

***Klebsiella pneumoniae* inoculum**

The *Klebsiella pneumoniae* used for the study was isolated, during routine abattoir inspection, from the lungs of a White Fulani cow presented for slaughter at the main abattoir in Mbaitoli Local Government Area of Imo State, Nigeria. Mbaitoli is situated between Owerri city (5.485°N 7.035°E) and Orlu town (05°47′47″N 07°02′20″E). The organism was identified using cultural (MacConkey agar) and biochemical characteristics (urease test, Citrate utilisation and Voges-Proskauer)

Four millilitres (4 ml) of Nutrient broth containing *Klebsiella pneumoniae* at 5.52 x 10^8 (cfu/ml) was introduced via nasal instillation (1 ml) and intra-tracheal route (3 ml) - under local anaesthesia of the ventral neck region using lignocaine; at days 0, 7 and 14 into goats in Groups II, Group III and Group IV respectively. The method used was intra-tracheal instillation method as described by Thrall *et al*.,(1978).

Bacteriology

Post-instillation, nasal swabs were taken after 48 h and thereafter on a daily basis for bacteriology until the bacteria can no longer be detected. Swabs were cultured in nutrient broth at 37^o C for 24 h for pre-enrichment and thereafter sub-cultured on MacConkey agar at 37^o C for 24 hrs.

Histopathology

Humanely sacrificed goats were necropsied. Samples of the lungs were fixed in 10 % buffered formal saline for at least 48 h, dehydrated in ascending concentrations of alcohol, cleared in xylene for 1 h 30 minutes, infiltrated and embedded in paraffin wax blocks. Sections 5 µ thick were cut and mounted on slides. The slides were stained with Hematoxylin, counter-stained with Eosin (H &E stains) and viewed under a light microscope. The method used was that described by Drury and Wellington (1967).

RESULTS

Re-isolation

In Grp I (Uninfected, negative control), *Klebsiella pneumoniae* was not isolated from nasal swabs of any of the goats in the group at any point throughout the experiment.

In Grp II (*Klebsiella pneumoniae*, positive control I), *Klebsiella pneumoniae* was not re-isolated from nasal swabs of goats in the group after 48 h post-instillation, and up to six days thereafter. Also, there was no isolation of the bacteria from the lungs at sacrifice, six days post-instillation.

In Grp III (*T. brucei + K pneumoniae*, acute), *Klebsiella pneumoniae* was not re-isolated from nasal swabs of goats in the group after 48 h post-instillation. Also, there was no isolation of *Klebsiella pneumoniae* from the lungs at sacrifice, six days post-instillation.

In Grp IV (*T. brucei + K pneumoniae*, sub-acute), *K. pneumoniae* was re-isolated from the nasal swabs of the three goats in the group at 48 h post-instillation. Two of the goats that tested positive for the bacteria died after six (6) days post-instillation and the bacteria were isolated from the lungs at necropsy (Fig 1).

Fig 1: *Klebsiella pneumoniae* culture from nasal swab of Grp IV on MacConkey agar. Note discrete large raised circular pinkish and mucoid colonies typical of *Klebsiella pneumoniae* on MacConkey agar

In Grp V (*T. brucei*, positive control II), *Klebsiella pneumoniae* was not isolated from nasal swabs of any of the goats in this group at any point throughout the experiment.

Pathology

Grossly, the lungs of group II goats appeared quite normal with no lesions apparent. However microscopically, there was evidence of mild inflammation of the interstitial walls, with infiltration by inflammatory cells; mostly mononuclear inflammatory cells. The interstitial architecture was disrupted as the pneumocytes became disorganised. There was very mild exudation into the alveoli. There were also areas of congestion. There were areas of atelectasis interspersed with areas of emphysema (Fig. 2).

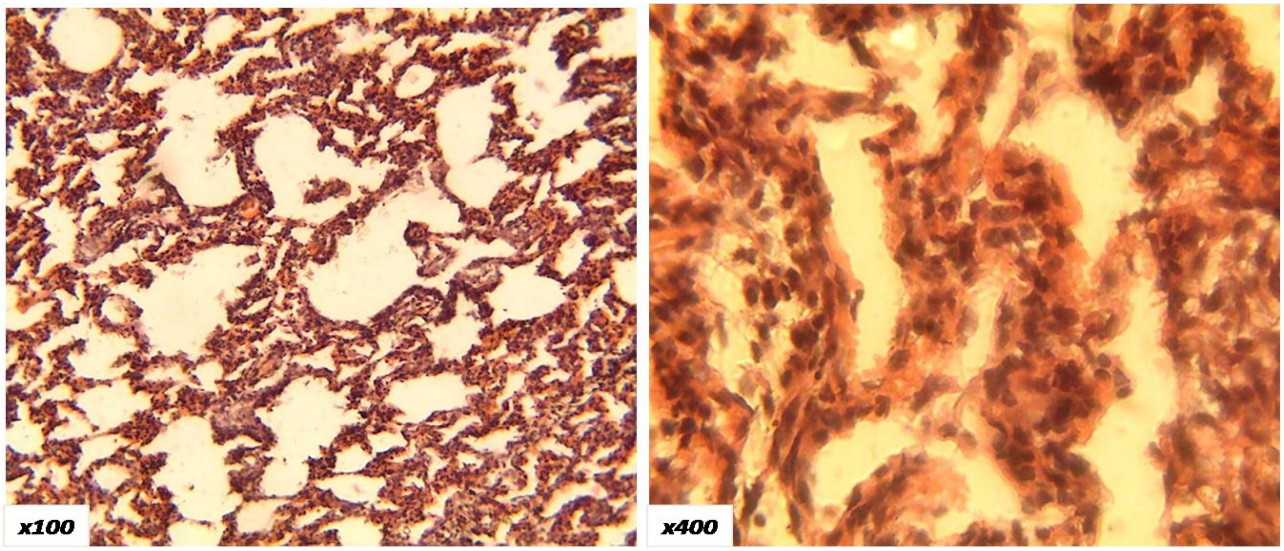

Fig 2: Histopathology of Grp II (single *K. pneumoniae* infection); there was atelectasis, inflammation of the interstitial septa and mild exudation into the alveoli. At higher magnification, note the disorganised pneumocytes and infiltration by inflammatory cells.

Grossly, the lungs of group III were similar to group II lungs, appearing normal with no apparent lesions except for areas of mild consolidation on the cranial lobes. Microscopically, there was mild to moderate inflammation of the interstitial walls, with presence of inflammatory cells; mostly mononuclear inflammatory cells and very few polymorphonuclears. The arrangement of the pneumocytes was also disorganised. There was mild exudation into the alveoli as well as mild sero-fibrinous exudation into the interlobular septa and pleura of the lungs (Fig. 3).There was also congestion and areas of atelectasis interspersed with areas of emphysema..

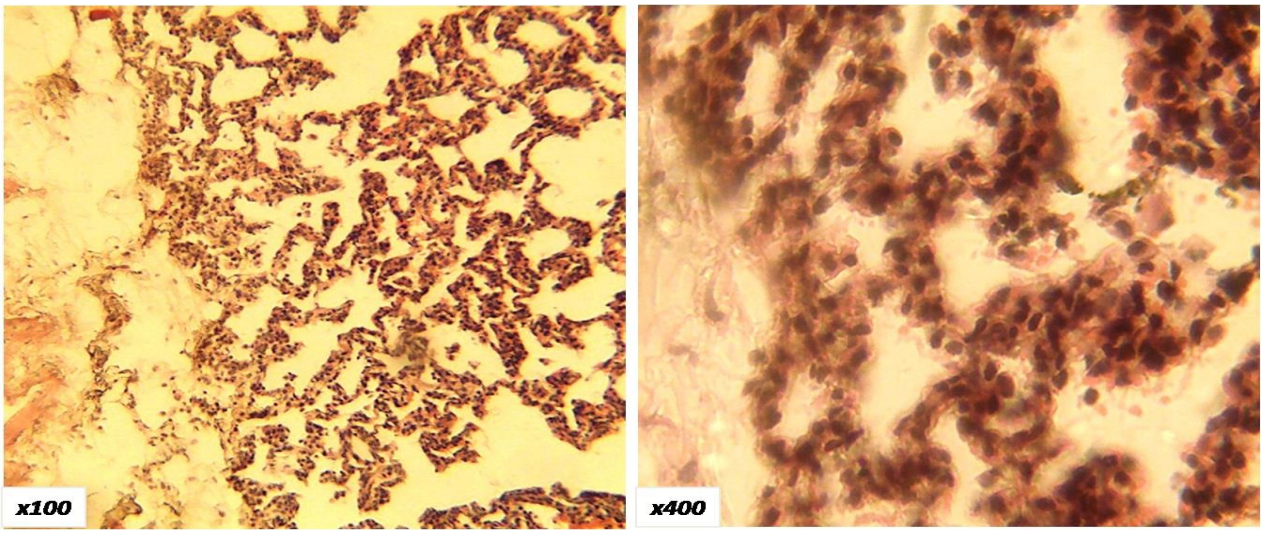

Fig 3: Histopathology of Grp III lungs (*T. brucie + K. pneumoniae* acute infection); note inflamed inter alveolar septa, presence of inflammatory cells and the sero-fibrinous exudation into the pleura. Higher magnification shows the inflammatory cells (mostly mononuclear) and pneumocytes actively reacting to the bacteria infection.

Grossly, group IV had multifocal and coalescing raised areas of abscessation and generalised fibrinous pleurisy on both lungs. There were also areas of red hepatisation on the ventral part of the lungs. Microscopically, the group IV lungs showed severe inflammation of the interstitial walls, with haemorrhage and massive presence of inflammatory cells - a lot of mononuclear and polymorphonuclear inflammatory cells. There was also severe suppurative exudation into the interlobular septa and fibro-purulent exudation into the pleura (pleuritis) (Fig. 4).

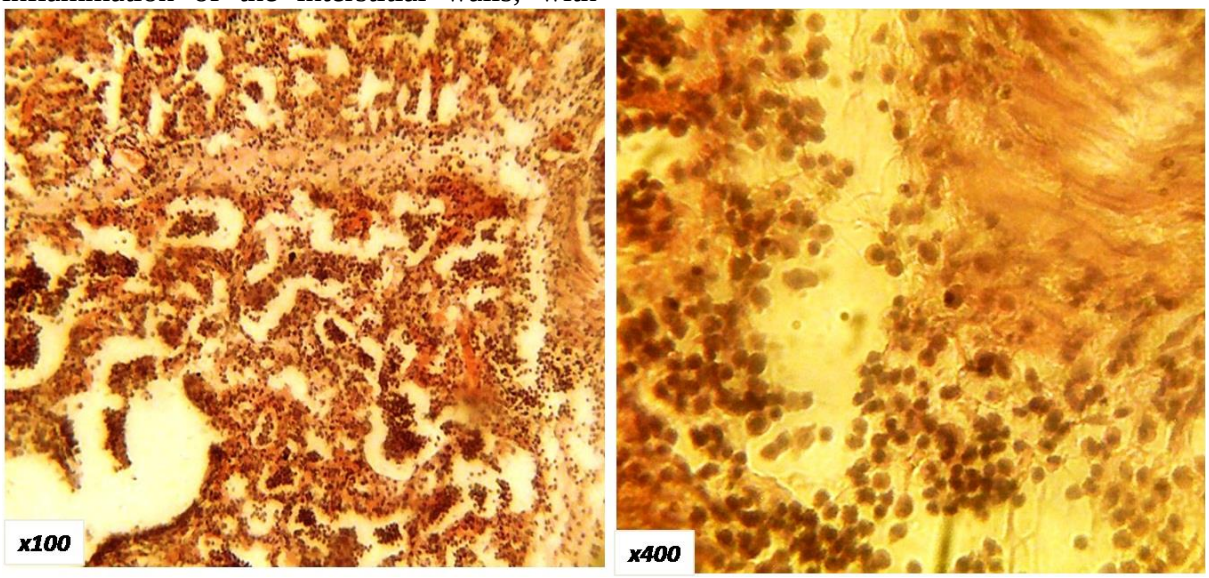

Fig 4: Histopathology of Grp IV lungs (*T. brucie + K. pneumoniae,* sub-acute infection); showing the massive inflammatory cell infiltrations into the alveoli and interlobular septa.

Grossly, group V showed generalised congestion and oedema. And microscopically, there was congestion as well as oedema fluid in the alveoli (Fig. 5).

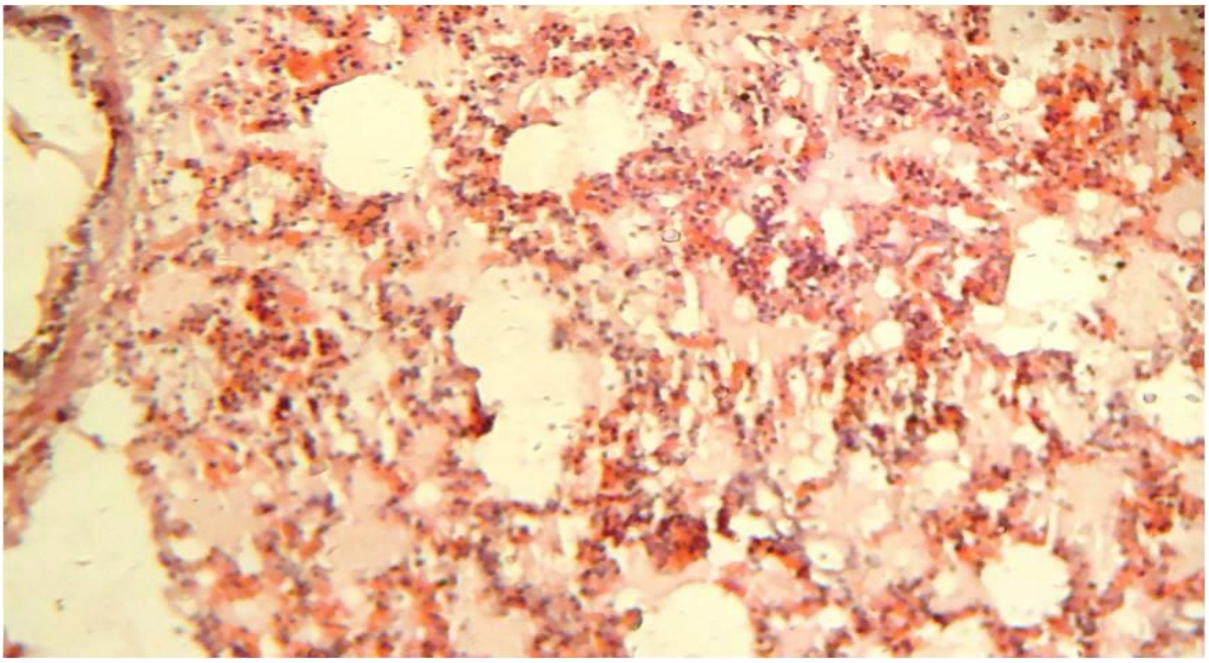

Fig 5: Histopathology of Grp V lungs (Single *T. brucie*infection); Note generalised congestion and oedema with mild cellular infiltration.

DISCUSSION

Klebsiella pneumoniae causes destructive changes in the lungs like necrosis, inflammation, haemorrhage and the formation of lung abscesses (Ningthoujam, 2011). The mild exudations seen in the lung sections of the Grp II goat could be as a result of the presence of particulate material in the inoculums with mild inflammatory response. It also occurred in Grp III. This could also be evidence of inflammatory response to the instilled bacteria as was the case in Grp III, where the greater distensions of the inter-alveolar tissue by exudates compared to Grp II goats was an indication of a greater inflammatory reaction. However, infection could still not establish in both groups. A functional defence mechanism in the upper respiratory tract as well as the mobilisation of the defensive inflammatory cells in the interstitium would have prevented establishment and spread of the infective bacteria within the lung parenchyma and so limited the severity of pathology. It is likely that the *Klebsiella pneumoniae* used in this study does not have the K antigen exposed at the cell surface. Previous studies had reported that phagocytosis is only impeded in *Klebsiella pneumoniae* infections involving serotypes that have the K antigen exposed at the cell surface because they are unable to activate complement (Merino *et al.,* 1992).

The bacterium *Klebsiella pneumoniae* in the presence of the *T. brucei* caused severe bronchopneumonia in Grp IV goats. The severe exudation into the pleura, the interlobular septa and the alveoli spaces seen in histologic lung sections of goats in Grp IV compared with Grp III suggests that while there was concurrent infection in both, the pathogenicity of the *Klebsiella pneumoniae* was potentiated by the sub-acute *T. brucei* infection of goats in Grp IV. The bronchopneumonia observed in this study agrees with previous report by Misone*t al.,* (1980) in mice.

Severe cases of *Klebsiella pneumoniae* infection in addition to lung tissue destruction, causes the formation of lung abscesses which can predispose to empyema of the thoracic cavity (Ningthoujam, 2011).

It would appear the defence mechanism of the respiratory tracts of goats in Grp IV were compromised by the *T. brucei* infection, hence the lungs were unable to effectively limit *Klebsiella pneumoniae* proliferation within and across the alveoli. *Klebsiella pneumoniae* infection in animals has been associated with immune suppression resulting from drugs, malnutrition, stress, endocrine

diseases, and other infections (Nelson *et al.*, 2003).

The lobar pneumonia reported in rats by Domenico *et al.*, (1982) and in mice by Yadav *et al.*, (2003) were observed under chronic conditions. However, in certain situations, lobular pneumonia readily develops into the lobar form of pneumonia if the affected animal does not die during the early stages of the disease.

The pulmonary congestion and severe oedema with mild inflammation of lung tissueas observed in lung sections of group V goats were obviously a result of the *T. brucei* infection in the absence of *Klebsiella pneumoniae*.

CONCLUSION

From the result of this study, the authors conclude therefore that *Klebsiella pneumoniae* isolated from cattle can under certain immune compromising conditions be pathogenic to West African Dwarf goats. While primary *Klebsiella pneumoniae* infections in clinically normal WAD goats may not readily lead to pneumopathies; and acute *Trypanosoma brucei* infection in WAD goats may not predispose to *Klebsiella pneumoniae* pneumopathies, sub-acute to chronic *Trypanosoma brucei* infections in WAD goats may however, predispose WAD goats to pulmonary lesions of *Klebsiella pneumoniae*. Single *Trypanosoma brucei* infections in the WAD goat may cause congestion in the inter-alveolar interstium and oedema in the lungs, concurrent infection with *Klebsiella pneumoniae* may lead to moderate to severe bronchopneumonia.

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