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Therapeutic management of sheep bacterial respiratory tract infections through aerosol route of antibiotic administration using nebulizer

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Abstract
Sheep showing clinical signs of nasal discharges, coughing, fever and dullness were tentatively diagnosed as respiratory tract infections were included in the present study which were confirmed as respiratory diseases of bacterial origin through cultural examination. Screened sheep which were positive for *Mannheimia haemolytica* were divided in to two groups for therapeutic study. Group-I (n=6) treated with ceftiofur sodium through standard intra muscular route and Group-II (n=6) nebulized with ceftiofur sodium diluted with normal saline (1:3) using piston type nebulizer @1.1 mg/kg bw for five days.
Sheep received nebulization therapy (Group-II) have shown significantly better clinical improvement and cure that of group –I which was evaluated in terms of remission of clinical signs, improved respiratory score and revival of haematological parameters like Hb, TLC and neutrophil count. Hence it is concluded that ceftofur sodium can be administered through aerosol route through nebulization in ailing sheep with promising results.

Keywords: sheep, broncho pneumonia, ceftiofur, aerosol route, nebulization

Introduction
Small ruminants are more susceptible to respiratory tract diseases due to their anatomical variation of lung alveoli. Respiratory infections alone contributes to 5.6 per cent of entire diseases and 23 per cent mortality is attributed to ovine respiratory disease complex [1].
Early detection of respiratory diseases and instituting efficient treatment are mandatory for control of infections and thus increases profitability to shepherds. Treatment of ailing animals and in contact sheep will significantly reduce the losses due to any infectious diseases particularly bacterial respiratory tract diseases [2].
Antimicrobial should reach the site of infection and should maintain in the vicinity for sufficient time and bind to target site and remain for sufficient length of time. But following parenteral or oral administration above two requirements are not fulfilled optimally as drug diffusion should take place from circulation to target tissue. Hence diffusion of antibiotic from systemic blood circulation may result in inefficient drug delivery to the lungs in case of respiratory diseases. So it is postulated that response to therapy might be improved by directly delivering the antibiotic to the site of infection via aerosol route by nebulization [3].
Many researchers attempted aerosol route of administration of antimicrobials in different species like calves [4, 5], dogs [6], horses [7] and in goats [8] successfully with promising results.
Advantages of aerosol administration of antimicrobials includes higher antimicrobial concentration in the respiratory tract, rapid onset of action, decrease in the total dose administration and avoidance of systemic side effects. Aerosol administration of antimicrobials shown higher efficacy in decreasing the severity or duration of infection [9].
Hence taking in to consideration of above aspects, the present study was carried out with an objective of therapeutic management of sheep bacterial respiratory tract infections through aerosol route of antibiotic administration using nebulizer.

Materials and Methods
Animals
Sheep ailing with respiratory signs like coughing, nasal discharges, pyrexia were included in the current study as tentatively suspected cases of mannheim pasteurellosis and later confirmed with cultural isolation and PCR.

Isolation and Identification of *Mannheimia haemolytica*

Nasal swabs from suspected cases of mannheim pasteurellosis were collected in a sterile manner and transferred to brain heart infusion broth (BHI broth) and incubated at 37 °C for 12hr. Loopful of overnight incubated broth culture is streaked on 5 per cent sheep blood agar and MacConkey's agar and incubated 37 °C for 24 hrs. Later these isolates were confirmed with PCR

Sheep suffering from bronchopneumonia were included in the current study based on respiratory score which was adopted from University of California-Davis respiratory disease scoring system with slight modifications (Table.1). The animals shown total clinical respiratory score more than five were included in the current study [10].

Sheep showing high respiratory score (>5) ailing with bacterial respiratory infections were randomly allocated in to two groups of six sheep (n=6) each. Group-I (n=6) is treated with ceftiofur sodium @ 1 mg/kg bw through standard intramuscular route for 5 days. Group-II (n=6) is administered with ceftiofur sodium @1mg/kg bw through aerosol route using piston compressor type nebulizer (Fig.1).Piston compressor nebulizer (Nureca, India) with modified face mask is fabricated and used for administration of antibiotic through aerosol route.

The calculated dose of ceftiofur sodium was placed in nebulizer cup, along with normal saline solution in the ratio of 1:3 [5]. The ceftiofur is administered using piston type nebulizer which generates the aerosol with 4 µ as maximum mass median diameter (MMD) at a pressure of three bar. The antibiotic in the form of aerosol was inhaled freely by sheep through a modified fabricated mask (Fig.2) for 15 minutes given in three episodes with a gap of 2-3 minutes.

Efficacy of treatment was evaluated in terms of resolution of clinical signs, speed of recovery signs (Respiratory score) and haemogram (TLC, TEC, Hgb, PCV and DLC).

Statistical Analysis

Data were entered into a Microsoft Excel spreadsheet, verified for correctness, and IBM-SPSS software version 23.0 was used to analyse the numerical data recorded in the present study. To know the significance of difference between the groups ANOVA was performed. Paired sample t-test was performed to compare before and after treatment. The results were analysed statistically as per the principles of Snedecor and Cochran (1994).

Table 1: Respiratory scoring system for selecting the sheep with bronchopneumonia

Clinical sign	Respiratory Score			
	0	1	2	3
Nasal discharges	None	Small amount unilateral cloudy	Bilateral cloudy or excessive mucous discharge	Copies, bilateral mucopurulent
Eye discharges	Normal	Small amount	Moderate, bilateral	Heavy
Head tilt/ droopy neck	Score :5			
Cough	None	Induced single cough	Induced repeated cough	Repeated spontaneous cough
Breathing :	Normal		Rapid & difficulty in breathing	
Rectal temperature	<103.5°F		>103.5°F	

Fig 1: Piston compressor type nebulizer

Fig 2: Sheep receiving ceftiofur sodium through nebulization (Aerosol administration)

Table 3: Effect of nebulization on haematological parameters

Sl. No.		Parameter	Day	Healthy	Group I	Group II
1		Respiratory score	Zero day	1.33±0.21^{a}	8.83±0.48^{b}	9.50±0.22^{b}
			3rd day	1.17±0.17^{a}	4.33±0.42^{b}	4.17±0.31^{b}
			7th day	1.50±0.22^{b}	1.83±0.17^{a}	0.67±0.21^{b}
2		Hemoglobin (g/dL)	Zero day	10.35±0.17^{b}	8.48±0.18^{a}	8.08±0.20^{a}
			3rd day	10.13±0.12^{b}	8.63±0.14^{a}	8.83±0.24^{a}
			7th day	10.10±0.07	9.82±0.13	10.25±0.23
3		PCV (%),	Zero day	29.50±0.76	28.92±0.27	29.52±0.39
			3rd day	29.33±0.88^{b}	26.67±0.42^{a}	30.00±0.73^{b}
			7th day	26.83±0.91^{a}	26.83±0.75^{a}	31.33±0.33^{b}
4		TEC ($x10^6$/µL),	Zero day	6.38±0.18^{b}	5.30±0.008^{a}	5.62±0.12^{a}
			3rd day	6.27±0.08^{c}	5.38±0.10^{a}	5.83±0.08^{b}
			7th day	6.32±0.09^{b}	5.77±0.06^{a}	6.15±0.07^{b}

5		TLC ($x10^3$/µL),	Zero day	10.00±0.08[a]	12.95±0.26[b]	13.35±0.22[b]
			3rd day	10.17±0.25[a]	11.35±0.27[b]	10.98±0.33[a]
			7th day	10.20±0.16[b]	9.87±0.23[b]	8.83±0.08[a]
6	Differential Leucocyte Count	Neutrophils (%)	Zero day	35.00±0.63[a]	60.17±0.75[b]	60.17±0.65[b]
			3rd day	34.17±0.31[a]	48.17±0.54[c]	40.00±1.03[b]
			7th day	33.00±1.03	34.00±2.91	29.00±0.26
		Lymphocyte (%)	Zero day	59.00±0.52[b]	32.50±0.76[a]	31.50±0.89[a]
			3rd day	60.67±.33[c]	47.33±0.80[a]	54.83±1.14[b]
			7th day	62.00±1.21	62.67±2.82	67.17±0.40
		Monocyte (%)	Zero day	4.00±0.26	5.83±0.75	6.17±0.65
			3rd day	3.50±0.22	3.50±0.50	3.50±0.34
			7th day	3.50±0.22[b]	2.33±0.21[a]	2.83±0.31[ab]
		Eosinophils (%)	Zero day	2.00±0.00[ab]	1.50±0.22[a]	2.17±0.17[b]
			3rd day	1.67±0.21[b]	1.00±0.00[a]	1.67±0.21[b]
			7th day	1.50±0.22[b]	1.00±0.00[a]	1.00±0.00[a]

The values with different superscript differ significantly at 0.05% level of significance

Table 3: Haematological parameters and respiratory score observed in sheep before and after therapeutic protocols using nebulization

Parameter	Group-I		Group- II	
	Before Treatment (Zero day)	After treatment (7th day)	Before treatment (Zero day)	After treatment (7th day)
TEC($x10^6$/ µL)	5.30±0.10*	5.77±0.06*	5.62±0.12	6.15±0.07*
Haemoglobin (g/dL)	8.48±0.18	9.82±0.13*	8.08±0.20	10.25±0.23*
PCV (per cent)	28.92±0.27	26.83±0.75	29.52±0.39	31.33±0.33*
Total leucocyte count ($x10^3$/ µL)	12.95±0.26	9.87±0.23*	13.35±0.22	8.83±0.01*
Neutrophils	60.17±0.75	34.00±2.91*	60.17±0.65	29±0.26*
Lymphocytes (%)	32.50±0.76	62.67±2.82*	31.50±0.89	67.17±0.40*
Monocytes (%)	5.83±0.75	2.33±0.21*	6.17±0.65	2.83±0.31*
Eosinophils (%)	1.67±0.21	1.67±0.21	1.50±0.22	1.00±0.00
Clinical score	8.83±0.48	1.83±0.17*	9.50±0.22	0.67±0.21*

*: Significant at $P < 0.05$ when compared to before therapy

Results and Discussion

In this current study clinical cases of sheep ailing with bacterial respiratory disease were divided in to two groups (n=6 each) and treated with ceftiofur sodium @1mg/kg through standard intra-muscular and nebulization route. It is found that aerosol route of administration was found to be effective in terms of remission of clinical signs, hematology when compared to intramuscular route regimen (Table 2, Fig, 3-7).

Respiratory score in nebulization treated sheep (group-II) was significantly improved when compared to intramuscular group by the seventh day of post treatment. Similar findings were reported [5] in calves suffering with BRD who studied recovery in terms of respiratory score and reported that calves showed normal clinical score by 10th day of post treatment through nebulization and endorsed better clinical outcome.

In the current study nebulization group showed quicker revival of haematological parameters to normal range when compared to standard intramuscular group. Reduction of Total Leucocyte Count and neutrophil count as well as improvement in TEC, Haemoglobin were found to be significant in animals those received ceftiofur sodium through aerosol route when compared to intramuscular route (Table 3). These findings are in accordance with the earlier researchers [5, 8].

Nebulization therapy results in high concentration of antimicrobials within short time in upper and lower respiratory tract and significantly reduces the mortality in calves and early recovery which are ailing with bovine respiratory disease [4, 5].

Administration of ceftiofur through nebulization is well tolerated and significantly higher drug concentration in lower respiratory tract when compared to administration of same dose by intramuscular route in horses [7].

Aerosol administration of gentamicin to normal horses results in drug concentration in bronchial lavage fluid 12 times that achieved after intravenous administration. Aerosolized ceftiofur sodium (1 mg/kg) is superior to intramuscular administration in treatment of calves with *Mannheimia haemolytica* [11].

Significant reduction in oxidative stress besides clinical improvement and haematological parameters revival in a study who administered moxifloxacin through aerosol route in caprine respiratory infections and reported efficient clinical outcome [8].

Hence it is noteworthy that aerosol administration of antibiotics through nebulizer will yield quick and higher drug concentration locally because of avoiding blood-bronchial barrier limitation, development multi drug resistance, lower systemic concentration thus less systemic toxicity and minimal side effects and usage of relatively low dose thus economical [12, 3, 13, 14, 15].

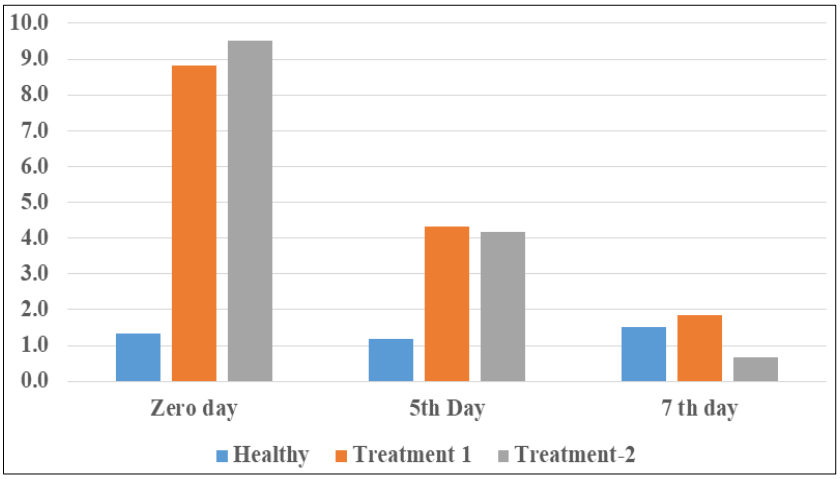

Fig 3: Effect of Nebulization -Mean respiratory score in treatment groups before and after treatment

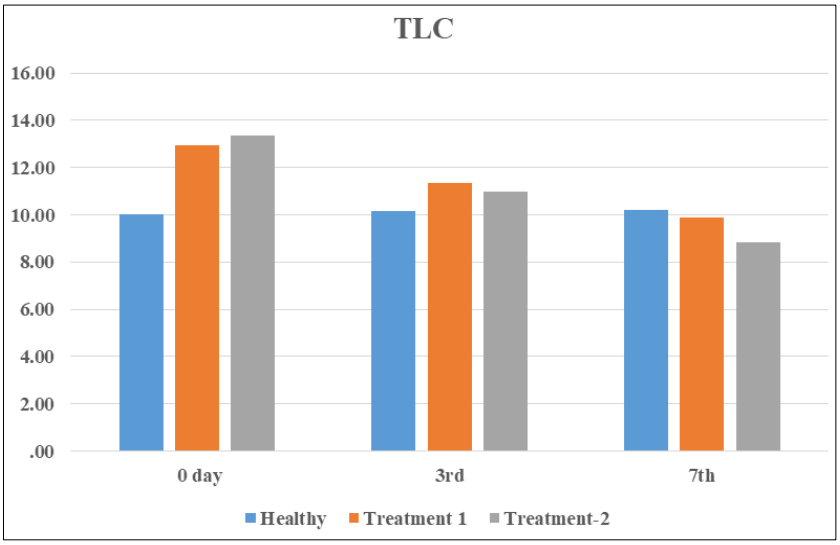

Fig 4: Effect of Nebulization -Mean TLC ($x10^3$/ µL) values in treatment groups before and after treatment

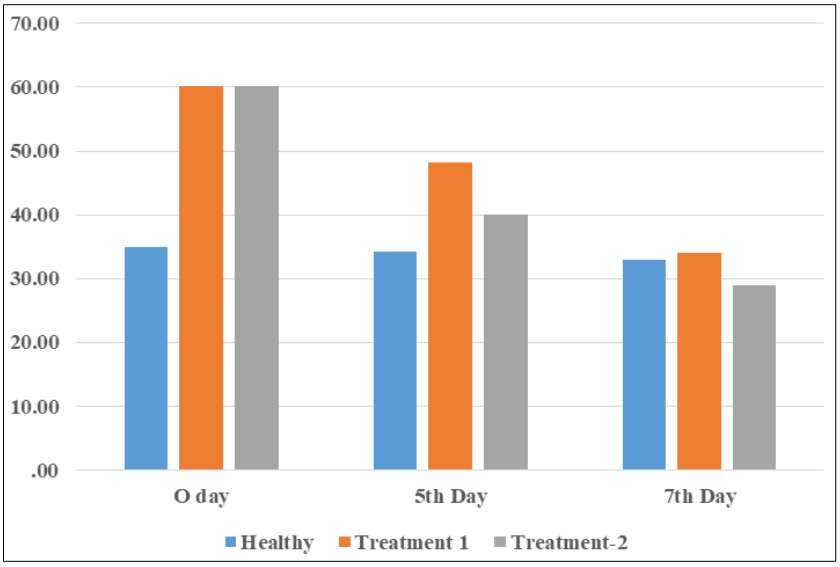

Fig 5: Effect of Nebulization- Mean Neutrophil count (%) values in treatment groups before and after treatment

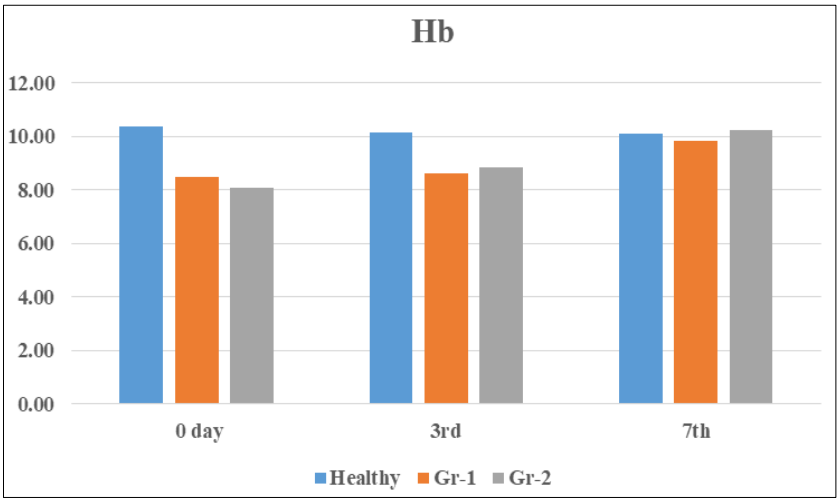

Fig 6: Effect of Nebulization -Mean Haemoglobin (gm/dl) values in treatment groups before and after treatment

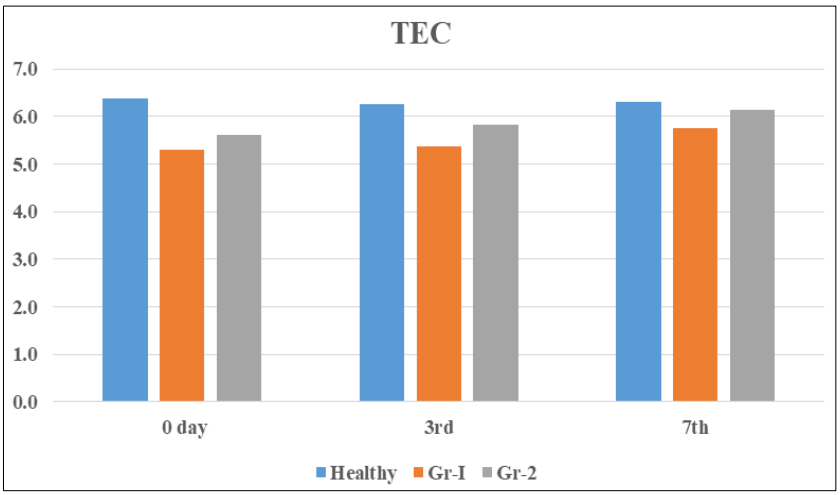

Fig 7: Effect of Nebulization- Mean TEC ($x10^6$/ µL) values in treatment groups before and after treatment

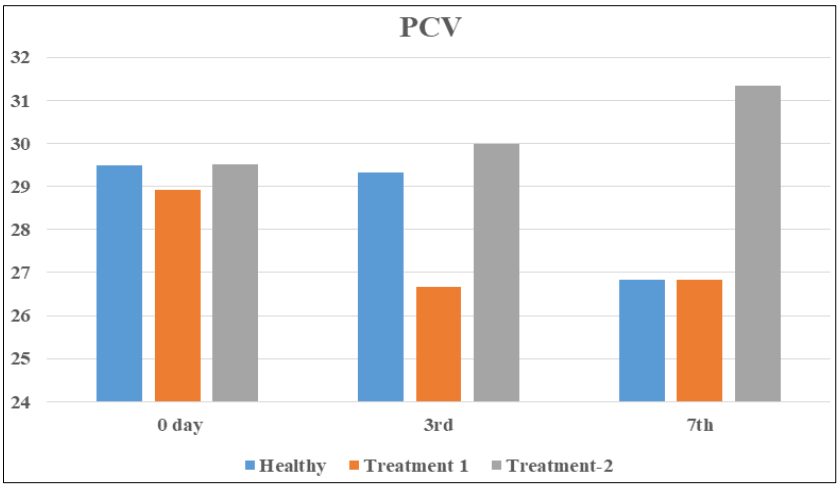

Fig 8: Effect of Nebulization- Mean PCV (%) values in treatment groups before and after treatment

Conclusion

Antimicrobial resistance is global problem and need of the hour to be addressed even in livestock health management which will indirectly develops drug resistance in human beings. Aerosol administration of ceftiofur sodium using piston type nebulizer will achieve good concentration in upper and lower respiratory tract and thus effectively used in sheep bacterial respiratory diseases conveniently without any adverse effects.

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References

1. Lacasta D, González JM, Navarro T, Saura F, Acín C, Vasileiou NGC. Significance of respiratory diseases in the health management of sheep. Small Ruminant Research 2019;181:99-102.
2. Garcia SR, Angelos JA, Row JD, Byrne BA, Wetzlich SE, Dana BS *et al*. Pharmacokinetics of ceftiofur crystalline-free acid following subcutaneous administration of a single dose to sheep, Am. J Vet. Res 2014;75(3):290-295.
3. Klepser ME. Role of nebulized antibiotics for the treatment of respiratory infections. Current opinion in infectious diseases 2004;17(2):109-112.
4. Sustronck B, Deprez P, Muylle E, Vermeersch H, Vandenbossche G, Remon JP. Evaluation of the nebulisation of sodium ceftiofur in the treatment of experimental *Pasteurella haemolytica* bronchopneumonia in calves. Research in veterinary science 1995;59(3):267-271.
5. Joshi V, Gupta VK, Dimri U, Kumar OV, Sharma DK, Bhanuprakash AG. Assessment of nebulisation of sodium ceftiofur in the treatment of calves naturally infected with bovine respiratory disease. Tropical animal health and production 2017;49(3):497-501.
6. Ayodhya S, Rao DST, Reddy YN, Sundar NS, Kumar VG. Therapeutic management of canine respiratory diseases of bacterial origin with nebulisation and suitable antibiotics in and around Hyderabad city, Andhra Pradesh. International Journal of Advanced Research 2014;2(4):656-662.
7. Fultz LM. Pharmacokinetics of ceftiofur crystalline free acid in adult horses after weekly intramuscular and subcutaneous administration and disposition of ceftiofur sodium after nebulization and intramuscular administration to weanling foals. Masters thesis submitted to University of Georgia, Athens 2014, 1-87.
8. Mandal RSK, Gupta VK, Joshi V, Kumar S, Bhanuprakash AG, Sharma DK *et al*. Therapeutic efficacy of aerosolized moxifloxacin in respiratory tract infection in goats. J Pharmacognacy and Phytochemistry 2019;8(3):125-129.
9. McKenzie HC. Characterization of Antimicrobial Aerosols for Administration to Horses Vetfolio 2019.
10. Love WJ, Lehenbauer TW, Kass PH, Van Eenennaam AL, Aly SS. Development of a novel clinical scoring system for on-farm diagnosis of bovine respiratory disease in pre-weaned dairy calves. Peer J 2014;2:e238.
11. Constable P, Hinchcliff KW, Done Stanley, Guenber W. Veterinary Medicine: A textbook of the diseases of cattle, sheep, pigs, goats, and horses, 11th ed., Saunders, Philadelphia, London 2016, 972-973.
12. Smith AL, Ramsey B. Aerosol administration of antibiotics. Respiration 1995;62(Suppl. 1):19-24.
13. Wood GC, Swanson JM. Aerosolised antibacterials for the prevention and treatment of hospital-acquired pneumonia. Drugs 2007;67(6):903-14.
14. Czosnowski QA, Wood GC, Magnotti LJ, Croce MA, Swanson JM, Boucher BA *et al*. Adjunctive aerosolized antibiotics for treatment of ventilator-associated pneumonia. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy 2009;29(9):1054-1060.
15. Cha ML, Costa LR. Inhalation therapy in horses. Veterinary Clinics: Equine Practice 2017;33(1):29-46.