Outbreaks of pneumonia in beef calves associated with bovine viral diarrhea virus seroconversion and other respiratory pathogens

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The present study describes the clinical, serological and bacteriolological findings in calves from two beef herds experiencing outbreaks of pneumonia. The clinical signs were nasal discharge, cough, pyrexia and increased respiratory rates. The morbidity and mortality rates over a month period were 40.72% and 15.63% respectively. Laboratory investigations revealed that bovine viral diarrhea virus (BVDV) was involved in and probably initiated both outbreaks as indicated by a significant increase in antibody titers against BVDV in sera of convalescent calves (paired serum samples). No antibodies bovine herpesvirus-1 (BHV-1), bovine respiratory syncytial virus (BRSV) and parainfluenza-3 (BPIV-3) viruses were detected in both acute and convalescent sera. *Mycoplasma bovis* was concurrently demonstrated in lungs of affected calves as it was isolated from 13 (81.25%) of examined lungs suggesting that there may be a synergism between bovine viral diarrhea virus and *Mycoplasma bovis* in the pathogenesis of pneumonia. A total of 15 (68.18%) isolates of *Mannheimia haemolytica*, 5 (22.73%) *Pasteurella multocida,* 1 (4.54%) *Pseudomonase aerugenosa,* 3 (13.64%) *Staphylococcus aureus*, 3 (13.64%) *Actinomycis pyogenes*, 1 (4.54%) *Klebsiella pneumonae*, 1 (4.54%) *Streptococcus pneumonae,* 2 (9.09%) *E. coli* and 2 (9.09%) *Aspergellus fumigatus* were recovered from lungs of calves suffering from pneumonia.

Enzootic calf pneumonia is one of the most common infectious diseases of beef calves. The term enzootic implies that the disease occurs continually within a herd and there is always risk of flare-up. The occurrence and severity of pneumonia depends on series of complex interactions between several different infectious agents, environmental factors and the immunological status of the calf (Uttenhal *et al.,* 1996 and Tegtmeler *et al.,* 1999).

Numerous viruses and bacteria can cause pneumonia in calves. BRSV and PI-3 virus are frequently associated with pneumonia in beef calves. For more than a decade, evidences have suggested that increased risk of pneumonia in feedlot cattle may be associated with acute BVD infection (Haines *et al.,* 2004). BVDV infection was found more frequently in cattle dying of pneumonia (O'Connor *et al.,* 2001). Pasteurella species are the most common bacteria associated with the disease. However it should be noted that most outbreaks are caused by mixed infections involving more than one organism (Uttenhal *et al.,* 1996).

Mycoplasma bovis is a major and often overlooked cause of calf pneumonia. Recently, there has been an emerging evidence that *Mycoplasma bovis*, is involved in a high proportion of death losses associated with a multi-systemic disease in feeder calves (Haines *et al.,* 2001 and Shahriar *et al,* 2002). Cases of antibiotic-resistant pneumonia and fibrinous polyarthritis in which *Mycoplasma bovis* and BVDV infection were frequently detected (Haines *et al.,* 2001 and Shahriare *et al.,* 2002). Animals with *Mycoplasma bovis* infection showed chronic bronchopneumonia with necrosis and bronchiectasis, which is sometimes associated with fibrinous to fibrous pleuritis. (Nicholas and Ayling, 2003)

The objectives of this study were to present clinical information and to describe microbiological as well as serological findings of two outbreaks of pneumonia occurred in feedlot herds at Alexandria, Egypt and to determine the possible cause or causes.

Material and methods

Animals. A syndrome of calf pneumonia was observed in two feedlot calf herds in Alexandria province. The calves of both herds were imported from Hungary in the same tribe and were distributed in two farms, the first farm has 700 calves and the second has 400 calves. The ages of calves ranged from 9-12 months.

Samples. Post mortem examination of both dead and slaughtered calves was conducted. Apparently non-autolysed lungs were collected from theses animals (22 samples). The samples were kept in ice till transported to the Laboratory and immediate culture was done.

Twenty Serum samples were collected from diseased animals during the acute stage of the disease and three weeks later from the same survived animals that survived the disease. Sera were kept frozen till used.

Bacteriological examination. Samples were inoculated onto nutrient broth, and then cultured on nutrient agar, blood agar and MacConkey agar plates. The inoculated plates were incubated at 37 ^{0}C for 48 hours. Suspected colonies were characterized on the basis of colony morphology and pure colonies were identified biochemically according to Koneman *et al.* (1983).

Cultivation of fungi was carried out on Sabouraud dextrose agar with 50 IU/ml penicillin and 0.05 mg/ml dihydrostreptomycin and the incubation of the plates was at 30°C. The species identification was based on morphological criteria according to Larone (1995).

Isolation and identification of mycoplasma organisms were carried out as described by (Tamada *et al.,* 2002). Specimens were homogenized (10% w/v) in PPLO broth (Difco Laboratories Detroit, MI, USA) supplemented with 15% horse serum, 2.5% yeast extract, 1mg/ml ampicillin, 0.02% thallium acetate, and 0.0024% DNA. Three serial 10-fold dilutions were then made in broth and subcultured on PPLO agar (Difco Laboratories), supplemented with the same additives in PPLO broth. Identification of mycoplasma was done using reference mycoplasmal antisera according to Clyde (1964) in Mycoplasma department, Animal Health Institute, Doki, Giza, Egypt

Virus neutralization test was carried out according to Rossiter *et al.* (1985). All sera were inactivated in water bath at 56 ^{0}C for 30 minutes and serially diluted in microtiter plates starting with dilution 4. An equal volume of BHV-1, BVDV, BPIV3 and RSV (100 $TCID_{50}$/50µl) were added to each well and incubated for one hour at 37^{o}C. Each well in the plate received 0.1ml of MDBK cell suspension containing 15000 cells. The plates were incubated for 7 days at 37^{o}C and microscopically examined daily for the development of cytopathic effect. The endpoint was expressed as the highest dilution of serum, which neutralized the virus.

Results

Epidemiological data. Two outbreaks of pneumonia were observed in two feedlot herds (9-12 months old). The cases started to appear in the farm one month after animal arrival from Hungary. Within a month, about 280 calves out of 700 showed clinical signs of pneumonia in the first farm (morbidity was 40%) and the mortality was13.90%. In the second farm the morbidity was 42% and the mortality was 18.45. (Table 1).

Clinical signs. Calves showed signs of dry, hacking cough, depression, fever, lacrimal and nasal discharges, reduced appetite, rapid respiration, labored breathing and mouth breathing. Some cases showed only cough and poor growth. These animals were chronically ill and failed to thrive, so they were eliminated from the herd because of lack of appropriate weight gain and failure to respond to treatment.

Gross pathology. Post mortem examination of either dead or slaughtered calves revealed that the great majority of lungs of some calves showed pneumonic lesions with catarrhal, mucopurulant or purulent exudates. The lungs contained also areas of suppuration. Most cases showed fibrinous pleuritis along with the pneumonia. The affected cranioventral areas of the lungs were markedly consolidated with raised, firm nodules on the pleural surface. On the cut surface, cavities that were 0.5 to 3.0 cm in diameter and filled with pale yellow necrotic materials were distributed throughout the parenchyma.

Paired serum samples. The results of the paired blood samples were recorded in terms of the presence or absence of a significant increase in titers. An increase between the first and second sample of at least four fold was considered significant. As shown in Table (1), there was significant increase in antibody titer to BVDV in 16 (80 %) of the examined samples. No significant increase in antibody titers was observed in cases of BHV-1, BRSV and BPIV3. Microbiological findings. Among the examined lungs (22), bacterial pathogens were isolated from all lungs (100%) (Table 3). *Mannheimia haemolytica* ((n= 15), *Pasteurella multocida* (n=5), *Pseudomonase aerugenosa* (n=1), *Staphylococcus aureus* (n=3), *Actinomycis pyogenes* (n=3), *Klebsiella pneumonae* (n=1), *Streptococcus pneumonae* (n=10) and *E.coli* (n=1) were found in 14 lungs, only one bacterial species was isolated. One lung was dually infected with *Mannheimia hemolytica* and *Actinomycis pyogenes*, two lungs were dually

Table (1): Morbidity and mortality of calves affected with pneumonia.

Farm	Total No. of calves	No. of clinical cases in a month	Morbidity rate	No of dead calves	Mortality rate
A	700	280	40	39	13.93
B	400	168	42	31	18.45
Total	1100	448	40.72	70	15.63

Table (2): Results of serological examination of acute and convalescent sera of calves (paired sera samples) against BVDV, BHV-1, RSV and PI3V.

Virus	No. of animals	Result of serology of paired sera samples		
		Increase *	Decrease**	No change***
BVDV	20	16 (80%)	0 (0%)	4 (20%)
BHV-1	20	0 (0%)	0 (0%)	20 (100%)
BRSV	20	0 (0%)	6 (25%)	14 (70%)
PIV3	20	0 (%)	0 (0%)	20 (100%)

* An increase between the first (acute) and second (convalescent) sample of at least four fold
** The titer of second sample is lower than that of first sample
*** There is no differences or there is increase between first and second samples less than 4 fold

Table (3): Microbiological findings in lung samples collected from pneumonic calves.

Bacterial isolates	No of tested samples	No positive samples	% of positive samples.
Mannheimia hemolytica	22	15	68.18
Pasteurella multocida	22	5	22.73
Pseudomonas aureugenosa	22	1	04.54
Staphylococcus aureus	22	3	13.64
Actinomycis pyogenes	22	3	13.64
Streptococcus pneumonae	22	1	04.54
Klebsiella pneumonae	22	1	04.54
Echerichia coli	22	2	9.09
Aspergellus fumigatus	22	2	9.09
Mycoplasma bovis	16	13	81.25

infected with *Mannheimia hemolytica* and *Staphylococcus aureus*. Two lungs were dually infected with *Mannheimia hemolytica* and *Pasteurella multocida*, one lung was dually infected with *Pasteurella multocida* and *Klebsiella pneumonae*, one lung was dually infected with *Mannheimia hemolytica* and *E. coli,* and one lung was infected with *Mannhemia hemolytica*, *Streptococcus pneumonae* and *E. coli. Aspergellus fumigatus* was isolated from two cases. *Mycoplasma bovis* was isolated from 13 out of 16 examined lung samples at a percentage of 81.25%.

Discussion

Bovine respiratory diseases are the most significant causes of mortality and elimination of calves from feedlots, and have great economic impact on cattle industry (Griffin, 1997). A recent study of calf pneumonia in feedlots has shown an increase in cases of antibiotic-resistant pneumonia (Haines *et al.,* 2001).

Two outbreaks of pneumonia were reported in feedlot herds in Alexandria province, many cases died and the diseased animals had poor response to the antibiotic treatment.

Serological examination of paired blood samples collected from diseased calves confirmed that the herds were BVDV infected as indicated by significant increase in antibody titers to BVDV in sera collected from convalescent animals. No antibodies were detected in the sera of convalescent and sick animals against RSV, BHV-1 and BPIV3

viruses. These results revealed that BVDV is able to induce primary respiratory disease in previously seronegative and immunocompetent calves. This finding demonstrated the role of BVDV infection in pneumonic calves. This agrees with earlier investigations, which suggest that high BVD antibody titers in cattle protect them from pneumonia (Booker *et al.,* 1999). While low antibody (Fulton *et al.,* 2002) or seroconversion after the arrival of calves to the feedlot is associated with increased occurrence of pneumonia (O'Connor *et al.,* 2001 and Martin *et al.,* 1990).

Immunohistochemical studies provided evidences that BVDV was present in tissue of many calves died from chronic unresponsive pneumonia and arthritis have been demonstrated by (Haines *et al.,* 2001 and Shahriar *et al.,* 2002). The ability of BVDV to induce primary respiratory disease has been a controversial issue. In addition, the immunosuppressive effect of BVDV has been considered determinant in facilitating secondary infections with potential respiratory tract pathogens such as BPIV3, BHV-1, or *Mannheimia hemolytica* (Potgieter *et al.,* 1984 and Potgieter, 1995). Field based evidence has indicated a primary role of BVDV in production of respiratory tract disease (Taylor *et al.,* 1997) that is supported by the finding of Baule *et al.,* (2001) who showed that this virus was able to induce a primary respiratory disease in infected calves.

Mycoplasma bovis was isolated from 13 out of 16 examined pneumonic lungs (81.25%) as shown in (Table 3) indicating a significant role of *Mycoplasma bovis* in the pneumonia outbreak. Ter Laak *et al.* (1992a, 1992b), in Netherlands, found *Mycoplasma bovis* in 20% of pneumonic lungs of fattening herds but only in a small number of apparently healthy calves. Following its introduction into the North and South of Ireland in 1994 from Mainland Europe, *Mycoplasma bovis* has been consistently isolated from 13 to 23% of pneumonic lungs (Brice *et al.,* 2000 and Byrne *et al.,* 2001). In France, *Mycoplasma bovis* was isolated from 30% of calf herds with pneumonia (Le Grand *et al.,* 2001) while in Britain, about 20–25% of pneumonic herds contained animals with antibodies to *Mycoplasma bovis* (Nicholas *et al.,* 2001). In the UK, significant antibody titers to *Mycoplasma bovis* were detected in nearly half of 55 examined pneumonic herds, of which only 7 herds had rising titers to viral pathogens: respiratory syncytial virus, infectious bovine rhinotracheitis or bovine viral diarrhea virus (Nicholas *et al.,* 2001). Tenk *et al.* (2004) found that mycoplasmas were isolated from 59.9% of pneumonic lung samples

Mycoplasma bovis on its own can induce mild pneumonia in calves, however, *Mycoplasma bovis* does not appear to induce severe respiratory disease enough to cause death and it is suggested that other microorganisms must have been involved in the death, probably in synergistic manner (Gourlay *et al.,* 1989). It is increasingly believed that *Mycoplasma bovis* is the predisposing factor in the infectious process leading to invasion by other bacterial pathogens possibly by compromising host defenses (Poumarat *et al.,* 2001 and Rebhun *et al.,* 1995). In a study of calves which had died of pneumonia, Buchvarova and Vesselinova (1989) showed that over a third of lungs were infected only with *Mycoplasma bovis* while the rest contained a combination of *Mycoplasma bovis* with *Pasteurella multocida* and / or *H. somnus.* These authors concluded that alterations in lungs were chiefly due to mycoplasma infection with the remaining bacteria contributing to complications in the pneumonic process.

The isolation of *Mycoplasma bovis* together with detection of BVDV infection in this study theorize, that there is synergistic action between BVDV and *Mycoplasma bovis* which was also supported by (Shahriare *et al.,* 2002) and (Haines *et al.,* 2001)

In the present study, *Mannhemia hemolytica* was isolated from the lungs at necropsy in (15 cases) Suggesting a synergism among *Mannhemia haemolytica*, *Mycoplasma bovis* and BVDV. However, 7 cases do not show *Mannhemia haemolytica* in bacterial culture. This may not reflect their true importance in the pathogenesis of the disease as it was reported by (Shoo, 1989). In that after intratracheal inoculation of this organism and the induction of sever pneumonia, the organism could not be isolated from the pneumonic lung in many cases. *Pasteurella multocida* was isolated from 5 cases. It was reported that *Pasteurella multocida* is less pathogenic in calves than *Mannhemia haemolytica* and its significance is difficult to be assessed (Gourly *et al.,* 1989).

Three isolates of *Actinomycis pyogenes* were detected in pneumonic lungs. It was reported that infection with other viral, bacterial or mycoplasmal agents usually precedes infection with *Actinomycis pyogenes* (Tigtmeler, *et al.,* 1999). Other bacteria that were isolated from

cases of pneumonia in this study including *Pseudomonas aerugenosa*, *Klebsiella pneumonia, Staphylococcus aureus, Streptococcus pneumonia* were also isolated from pneumonic calves by several authors (Ismail *et al.,* 1993 and El-haenaeey *et al.,* 1994).

In conclusion, these data revealed that. BVDV is able to induce respiratory disease in seronegative and immunocompetent calves. This finding demonstrates the importance of BVDV immunity in the protection of feedlot calves from pneumonia and reemphasize the need to address improved BVDV vaccination in feedlot calves. The results suggest that there may be synergism between bovine viral diarrhea virus and *Mycoplasma bovis* in induction of calf pneumonia. *Mycoplasma bovis* enhances the severity of pneumonia in beef calves and our results suggest that *Mycoplasma bovis* should be considered as a principal pathogen in chronic unresponsive pneumonia of feedlot cattle Antimycoplasmal drugs should be included in treatment of cases of pneumonia

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References

Baule, C.; Kulcs, G.; Bel, K.; Albert, M. and Mittelholzer, C. (2001): Pathogenesis of primary respiratory disease induced by isolates from a new genetic cluster of bovine viral diarrhea virus type I. J. Clin. Microbiol. 39 (1): 146-153.

Brice, N.; Finlay, D.; Bryson, D. G.; Henderson, J.; Mcconnell, W. and Ball, H. J. (2000): Isolation of *Mycoplasma bovis* from cattle in Northern Ireland 1993-1998. Vet. Rec. 146: 643-644.

Buchvarova, Y., Vesselinova, A. (1989): On the aetiopathogenesis of mycoplasma pneumonia in calves. Archiv fur Experimentelle Veterinarmedizin 43: 685-689.

Byrne, W. J.; McCormack, R.; Brice, N.; Markey, B. and Ball, H.J. (2001): Isolation of *Mycoplasma bovis* from bovine clinical samples in the Republic of Ireland. Vet. Rec. 148: 331-333.

Booker, C. W.; Guichon, P. T.; Jim, G. K.; Schunicht, O. C.; Harland, R. J. and Morley, P. S. (1999): Seroepidemiology of undifferentiated fever in feedlot calves in western Canada. Can. Vet. J. 40: 40-48.

Clyde, W.A. (1964): Mycoplasmal species identification based upon growth inhibition by specific antisera. J. Immunol., 92: 958-965.

Haines, D. M.; Moline, K. M.; Sargent, R. A.; Campbell, J. R.; Myers, D. J. and Doig, P. A. (2004): Immunohistochemical study of *Hemophilus somnus, Mycoplasma bovis*, *Mannheimia hemolytica,* and bovine viral diarrhea virus in death losses due to myocarditis in feedlot cattle. Can. Vet. J.; 45 (3): 231–234.

El-Haenaeey, A.; Mahmoud, A. M. and Haroun, E. M. (1994): Bacterial isolates from the respiratory tract of cattle and their zoonotic importance in AL-Quassem, Saudia Arabia. Vet. Med. J. Giza., 42: 189-196.

Fulton, R. W.; Cook, B. J.; Step, D. L. (2002): Evaluation of the health status of calves and the impact on feedlot performance: assessment of retained ownership program for post weaning calves. Can. J. Vet. Res. 66: 173-180

Gourly, R. N.; Thomas, L. H. and Wyld, S. G. (1989): Increased severity of calf pneumonia associated with the appearance of *Mycoplasma bovis* in a rearingherd. Vet. Rec., 124: 420-422

Griffin, D. (1997): Economic impact associated with respiratory disease in beef cattle. Vet. Clin. North. Am. Food Anim. Pract. 13: 367-377

Haines, D. M.; Martin, K. M.; Clark, E. G.; Jim, G. K. and Janzen, E. D. (2001): The immunohistochemical detection of feedlot cattle with chronic unresponsive respiratory disease and/or arthritis. Can. Vet. J. 42: 857-860.

Ismail, M.; Eljakee, J.; Attia, S. A.; Bagwaata, A. and Shoukry, S. (1993): Bacterial causes of respiratory disorders in buffalo calves in Egypt. Vet. Med. J. Giza, 41: 95-99.

Koneman, E. W.; Aan, S. D.; Dowell, V. R. and Sommers, H. M. (1983): Color atlas and textbook of diagnostic microbiology. 2nd ed., T.B. Lippencott, Co., New York, London.

Larone, D. H. (1995): Medically important fungi, a Guide to Identification., 3rd ed. ASM Press, Washington.

Le Grand, D.; Phillippe, S.; Calavalas, D.; Bezille, P. and Poumarat, F. (2001): Prevalence of *Mycoplasma bovis* infection in France. In: Poveda, J.B., Fernandez, A., Frey, J., Johnansson, K. E. (eds.), Mycoplasmas of ruminants: pathogenicity, diagnostics, epidemiology and molecular genetics, vol.. Europ. Comm., Brussels, 5: 106-109.

Martin, S. W.; Bateman, K. G.; Shewen, P. E.; Rosendal, S.; Bohac, J. G. and Thorburn, M. (1990): A group level analysis of the associations between antibodies to seven putative pathogens and respiratory disease and weight gain in Ontario feedlot calves. Can. J. Vet. Res. 54: 337.

Nicholas, R.A.J.; Wood, E.; Baker, S. and Ayling, R. D. (2001): Mycoplasmas isolated from ruminants in Britain 1995-2000. In: Poveda, J.B., Fernandez, A., Frey, J., Johansson, K. E. (eds.), Mycoplasmas of ruminants: pathogenicity, diagnostics, epidemiology and molecular genetics, vol. 5. European Commission, Brussels, pp. 116-120.

Nicholas, A. J. and Ayling R. D. (2003): *Mycoplasma bovis,* disease, diagnosis and control Review. Res. Vet. Sci. 74: 105-112

O'Connor, A.; Martin, S. W.; Nagy, E.; Menzies, P. and Harland, R. (2001): The relationship between the occurrence of undifferentiated bovine respiratory disease and titer changes to bovine corona virus and bovine viral diarrhea virus in three Ontario feedlots. Can. Vet. J. 65: 137-142.

Poumarat, F.; Le Grand, D.; Philippe, S.; Calavas, D.; Schelcher, F.; Cabanie, P.; Tessier, P. and Navetat, H.

(2001): Efficacy of spectinomycin against *Mycoplasma bovis* induced pneumonia in conventionally reared calves. Vet. Microbiol., 80: 23-35.

Potgieter, L. N. D.; McCracken, M. D.; Hopkins, F. M.; Walker, R. D. and Guy, J. S. (1984): Experimental production of bovine respiratory tract disease with bovine viral diarrhea virus. Am. J. Vet. Res., 45: 1582-1585

Potgieter, L. N. D. (1995): Immunology of bovine viral diarrhea virus. Vet. Clin. North. Am. Food Anim. Pract., 11: 501-520

Rebhun, W. C.; Guard, C. and Richards, C. M. (1995): Respiratory diseases. In: Diseases of Dairy Cattle. Williams and Wilkins, Baltimore, USA, pp. 79-80.

Rossiter, P. B.; Jessett, D. M. and Taylor, W. P. (1985): Microneutralization test for use with different strains of peste des petits ruminants virus and rinderpest virus. Trop. Anim. Health. Prod., 17: 75-81.

Shahriar, F. M.; Clark, E. G.; Janzen, E.; West, K. and Wobeser, G. (2002): Co infection with bovine viral diarrhea virus and *Mycoplasma bovis* in feedlot cattle with chronic pneumonia. Can. Vet. J., 43: 863-868.

Shoo, M. K. (1989): Experimental bovine pneumonic pasteurellosis A review. Vet. Rec., 124: 141-144.

Tamada, Y.; Ohura, K.; Kato, K.; Kimura, K. and Maeda, T. (2002): *Mycoplasma bovis* infection in calves with ear droops. J. Hokkaido Vet. Med. Assoc., 46: 308-311.

Taylor, L. F.; Janzen, E. D.; Ellis, J. A.; Van Den Hurk, J. V. and Ward. P (1997): Performance, survival, necropsy, and virological findings from calves persistently infected with the bovine viral diarrhea virus originating from a single Saskatchewan beef herd. Can. Vet. J., 38: 29-37

Tenk, M.; Stipkovits, L. and Hufnagel, L. (2004): Examination of the role of *Mycoplasma bovis* in bovine pneumonia and a mathematical model for its evaluation Acta. Vet. Hung., 52 (4): 445-56.

Ter Laak, E. A.; Noordergraaf, J. H. and Dieltjes, R. P. J. W. (1992a): Prevalence of mycoplasmas in the respiratory tracts of pneumonic calves. J. Vet. Med. Ser., 39: 553-562.

Ter Laak, E. A.; Noordergraaf, J. H. and Boomsluiter, E. (1992b): The nasal mycoplasmal flora of healthy calves and cows. J. Vet. Med., Ser., 39: 610–616.

Tigtmeler, C.; Uttenthal, A.; Friis, N.F.; Jensen, N. E. and Jensesn, H. E. (1999): Pathological and microbiological studies on pneumonic lungs from Danish calves. J. Vet. Med., 46: 693-700.

Uttenhal, A.; Jensen, P. B. and Blom, Y. (1996): Viral etiology of enzootic pneumonia in Danish dairy herds. Diagnostic tools and epidemiology. Vet. Rec., 139: 114-117.