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Isolation and Characterization of Respiratory Tract Bacterial Species from Domestic Animals with Pneumonic Lungs from Elphora Abattoir, Ethiopia

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Abstract: For this study pneumonic lung tissue and tracheal swabs were collected from sheep, goat and cattle slaughtered at Elphora abattoir. From the total of 170 bacterial isolates from sheep lung tissue, 10.6% *Bacillus* species, 14.7% *Mannheimia haemolytica* and 42.4% *Staphylococcus* species were isolated aerobically. The tracheal cultures also indicated that 5.8% were *Enterobacter* species, 13% were *Bibersteinia trehalosi* and 66.7% were identified as *M. haemolytica* from the total of 69 aerobic cultures. Out of 192 bacteria isolated from aerobic inoculation of goat lung; 6.3% were *M. haemolytica* species, *Micrococcus* species 12.5%, *Bacillus* species, 19.8% and *Staphylococcus* 45.3%. The tracheal culture of goat showed *Bacillus* species 26.5%, *M. haemolytica* 27.7% and *Enterobacter* species 30.1%. Eight *M. haemolytica* species were among 93 bacterial isolates of aerobic cultures of cattle lung followed by *Bacillus* species, 17 and *Staphylococcus* species, 29. While *M. haemolytica, B. trehalosi* and *Staphylococcus* accounted for 8, 14 and 18 out of the total of 57 tracheal cultures respectively. A total of 522 bacterial isolates were detected via anaerobic culture, in which 6.2%% of the 131 isolates from sheep lung were *Bacillus* species, 25.4% were *M. haemolytica* and 40% were *Staphylococcus* species while *B. trehalosi* and *Micrococcus* species took 5.4% each from the total isolates. Whereas, 12.7% *Enterobacter,* 19.7% *B. trehalosi* and 60.6% *M. haemolytica* species were isolated from a total of 71 sheep trachea culture isolates. Among anaerobic cultures of goat lung tissue *M. haemolytica* species accounts for 11.2% followed by *Bacillus* species, 25.6% and *Staphylococcus* 36% out of the total 125 bacterial species. In the tracheal culture *Enterobacter* counts for 7 followed by *Bacillus* species, 14 and *M. haemolytica* species, 33 from 61 isolates. 7.8% *B. trehalosi,* 23.4% *Bacillus* species and 42.9% *Staphylococcus* were isolated species from the lung of the cattle, while from the trachea 25.0% *M. haemolytica* and *Staphylococcus* each were isolated anaerobically.

Key words: Abattoir · Aerobic Bacteria · Anaerobic Bacteria · Respiratory Tract · Ruminant

INTRODUCTION

Ethiopia is home for diverse indigenous livestock, parallel to its diverse ecology, production systems and ethnic communities. The estimates of total livestock population are 50 884 005, 25 979 919 and 21 960 706 for cattle, sheep and goats, respectively [1]. According to FAO [2], the total annual meat production comes from cattle (63%), sheep (25%) and goats (12%). Although ruminants represent a great resource for Ethiopia, the rate of productivity per animal is low. The levels of foreign exchange earnings from livestock and livestock products are also much lower than would be expected hence; the current of contributions of the livestock sector in Ethiopia, at either the macro or micro level is below potential given the size of the livestock population [3]. Disease and poor animal management is largely responsible for this reduced productivity [4].

The impact of respiratory disease is extensive and can be measured as the sum of the direct economic losses occurring due to mortality, morbidity, treatment, prevention costs and loss of production (reduced animal performance and carcass quality) and the indirect costs like labor, infrastructure and intangibles [5]. Pneumonia has been noted to be the most prominent infectious cause of mortality, both on-farm and on-station, in Ethiopian

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[6, 7]. The causes of mortality due to respiratory diseases in the country are multi-factorial. Hence, the term respiratory disease complex (RDC) is used for the condition conventionally known as bronchopneumonia. RDC is a polymicrobial infection that develops when the immune system of the animal is compromised by stress factors such as crowding, transportation, draught and inclement weather combined with increased exposure to pathogens, may lead to respiratory infection. The importance of bacterial–viral synergism in RDC has been recognized for a long time [8]. Concurrent respiratory infestations by viruses (parainfluenza-3, reovirus, adenovirus and respiratory syncytial virus), *Mycoplasma* species (*M. ovipneumoniae*, *M. arginini*, *M. agalactiae* and others), lungworms (particularly *Dictyocaulus filaria)* and bacteria like *Mycobacterium, Fusobacterium*, *Arcanobaterium, Pasteurella multocida, Mannhemia haemolytica, Chlamydia psittacci*, *Bordetella parapertusis* can suppress the animal's immune system and inflict damage on the pulmonary tissues of most domestic animals allowing opportunistic microorganisms to colonize the lung and cause the disease [9].

In the country, there are high rates of mortality and morbidity following respiratory distress. Since, outbreaks of respiratory diseases occur frequently, killing significant numbers of livestock, hence, respiratory diseases are a great economic concern for livestock producers in Ethiopia [10].

Nonetheless, it is becoming increasingly difficult to make an etiological diagnosis because, although a single agent may be a primary invader, when the local resistance of respiratory mucosa is lowered, bacteria growing in the nose and throat extend downwards; usually producing multiple bacterial infections [11]. Besides, most of the infectious agents that cause respiratory disease are ubiquitous in nature and are normal inhabitants of the nasopharynx [12]. Although losses due to pneumonia have been widely reported to be high in Ethiopian highlands [6-7, 13-16], specific causative agents of the disease have not been systematically studied.

The importance and contribution of each respiratory bacterial species for the occurrence of RDC in ruminants, the country is not well documented yet. In addition, recent progress in epidemiologic surveillance and molecular biology has allowed the rapid recognition and identification of several newly emerging respiratory pathogens. The contribution of most of these bacteria in the occurrence of RDC is still undercover.

Therefore, the objective of this study was:

- To isolate and characterize respiratory tract bacterial species from trachea and lungs of sheep, goat and cattle with pneumonic lungs.

MATERIALS AND METHODS

Sample Collection and Transportation: Samples of pneumonic lung tissue and tracheal swabs were collected from cattle, sheep and goats at post-mortem for microbiological culture from Elphora abattoir enterprise. Each piece of tissue was placed in a separate sterile screw-capped universal bottle. Containers were fully labeled with the date, tissue and animal identification. Sterile instruments (knife, scalpel, forceps and scissors) were used for collecting specimens for microbiological culture, while swabs were submitted in tryptose soya broth media. After collection, the tracheal swabs were incubated immediately at 37°C for 24 hr both aerobically and anaerobically, while the lung tissues were processed immediately.

Isolation and Identification of Bacterial Species: The outer surfaces of the lungs were first seared with a heated spatula before the cut inner surface of the lungs were minced by sterile scissors and forceps. The minced tissue was inoculated into tryptic soya broth and incubated at 37°C for 24-48 hr both aerobically and anaerobically. After the first incubation the sample was streaked on 7% sheep blood agar and incubated at 37°C for another 24-48 hr both, aerobically and anaerobically.

The plates were then examined for presence of growth and colony morphology, size, shape and presence or absence of haemolysis. After taking note of cultural growth characteristics, subcultures were subjected to Gram's staining to study staining properties and cellular morphology under 100X objective of light microscope. Mixed colonies and Gram-negative bacteria were sub-cultured on both blood and MacConkey agars and incubated aerobically as well as anaerobically for further 24 hr. Pure cultures of single colony type, from both blood and MacConkey agars, were transferred onto nutrient agar-slants for a series of biochemical tests including catalase, oxidase and oxidative-fermentative (OF) tests for final identification, following standard procedures [17].

Data Analysis: The results of the collected data were entered in to Microsoft Excel spread sheet and finally were analyzed using SPSS version 15 software. Descriptive statistics was used to summarize the data generated from the study. The relative abundance of each species/genera was expressed as a percentage in comparison to the total number of isolates. In addition, chi-square test is computed in order to observe a relationship between the variants.

RESULTS

Isolation and identification of bacterial species from the respiratory tract of cattle, sheep and goats (abattoir survey).

Morphology, Staining and Cultural Characteristics of the Bacterial Isolates: In this study a total of 1186 bacteria were isolated from both aerobic and anaerobic cultures of pneumonic lung and trachea of ruminants on abattoir based study. In general, fourteen different bacterial genera including *Histophilus, Bordetella, Pasteurella, Mannheimia, Bibersteinia, Staphylococcus, Streptococcus, Micrococcus, Enterococcus, Arcanobacterium, Actinobacillus, Bacillus, Enterobacter, Klebsiella* and *Pseudomonas* were isolated.

Isolation Rate of Bacteria from Lung and Trachea of Ruminants from Aerobic Cultures: The data revealed that 12 different species of bacteria were isolated from 50 sheep lung tissue cultured aerobically and the higher percentage were taken by *Staphylococcus* species 72 (42.4%), *M. haemolytica* 25 (14.7%) and *Bacillus* species 18 (10.6%) out of the total of 170 isolates. While from tracheal culture 6 species of bacteria were recognized aerobically and it is pointed out that 46 (66.7%) were *M. haemolytica*, 9 (13%) were *B. trehalosi* and 6 (8.7%) were *Bacillus* species out of the total 69 isolates (Table 1).

Out of 192 overall isolates 8 species of bacteria were identified from aerobic inoculation of goat lung and 87 (45.3%) were *Staphylococcus* species followed by *Bacillus* species, 38 (19.8%) and *Micrococcus* species, 24 (12.5%) and the rest included *M. haemolytica* which accounted for 12 (6.25%) of the total isolates. From the aerobic cultures of trachea 6 species of bacteria were recognized and the highest percentage was attributed to *Enterobacter* species, 25 (30.1%) followed by *M. haemolytica,* 23 (27.7%) and *Bacillus* species 22 (26.5%).

Staphylococcus species, 29 (31.2%) were the highest among the 12 different bacterial species isolated from aerobic cultures of cattle lung which was followed by *Bacillus* species, 17 (18.3%) and *M. haemolytica* 8 (8.6%) out of the entire 93 isolates. While *Staphylococcus*, *B. trehalosi, M. haemolytica* and *Bacillus* species were among the 8 bacterial species identified and accounted for 18 (31.6%), 14 (24.6%), 8 (14%) and 8 (14%) respectively from the total of 57 subcultures.

The data showed that there is a statistically significant difference between each species of bacteria and anatomical site at $p < 0.05$. And the statistical significance was also observed between the species of animal and bacterial species at $p< 0.05$.

Table 1: Bacterial species isolated from lung and trachea of ruminants cultured aerobically

	Animal species and Anatomical site examined						
Bacterial species	Sheep Lung	Sheep Trachea	Goat Lung	Goat Trachea	Cattle Lung	Cattle Trachea	Total
Staphylococcus spp.	72 (42.4%)	3 (4.3%)	87 (45.3%)	6 (7.2%)	29 (31.2%)	18 (31.6%)	215 (32.4%)
Streptococcus spp.	6 (3.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	5 (5.4%)	3 (5.3%)	14 (2.1%)
Micrococcus spp.	8 (4.7%)	1 (1.4%)	24 (12.5%)	0 (0.0%)	7 (7.5%)	0 (0.0%)	40 (6.0%)
Bacillus Spp.	18 (10.6%)	6 (8.7%)	38 (19.8%)	22 (26.5%)	17 (18.3%)	8 (14.0%)	109 (16.4%)
M. haemolytica	25 (14.7%)	46 (66.7%)	12 (6.3%)	23 (27.7%)	8 (8.6%)	8 (14.0%)	122 (18.4%)
P. multocida	0 (0.0%)	0 (0.0%)	3 (1.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (0.5%)
B. trehalosi	7 (4.1%)	9 (13%)	0 (0.0%)	0 (0.0%)	5 (5.4%)	14 (24.6%)	35 (5.3%)
Enterococcus spp.	7 (4.1%)	0 (0.0%)	5 (2.6%)	0 (0.0%)	6 (6.5%)	0 (0.0%)	18 (2.7%)
Pseudomonas spp.	5 (2.9%)	0 (0.0%)	0 (0.0%)	1 (1.2%)	3 (3.2%)	0 (0.0%)	9 (1.4%)
Bordetella spp.	3 (1.8%)	0 (0.0%)	1 (0.5%)	0 (0.0%)	4 (4.3%)	0 (0.0%)	8 (1.2%)
Enterobacter spp.	4 (2.4%)	4 (5.8%)	10 (5.2%)	25 (30.1%)	4 (4.3%)	3 (5.3%)	50 (7.5%)
Klebsiella pneumoniae	2 (1.2%)	0 (0.0%)	0 (0.0%)	6 (7.2%)	0 (0.0%)	2 (3.5%)	10 (1.5%)
Actinobacillus ligneresii	2 (1.2%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (3.2%)	1 (1.8%)	6 (0.9%)
Arcanobacterium pyogens	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (1.1%)	0 (0.0%)	1 (0.2%)
Unidentified	11 (6.5%)	0 (0.0%)	12 (6.3%)	0 (0.0%)	1 (1.1%)	0 (0.0%)	23 (3.5%)
Total	170	69	192	83	93	57	664

Table 2: Bacterial species isolated from lung and trachea of ruminants cultured anaerobically

	Animal species and Anatomical Site						
Bacterial species	Sheep Lung	Sheep Trachea	Goat Lung	Goat Trachea	Cattle Lung	Cattle Trachea	Total
Staphylococcus spp.	52 (39.7%)	2 (2.8%)	45 (36.0%)	6 (9.8%)	33 (42.9%)	15 (25.0%)	153 (29.3%)
Streptococcus spp.	1 (0.8%)	0 (0.0%)	2 (1.6%)	0 (0.0%)	3 (3.9%)	1 (1.7%)	7 (1.3%)
Micrococcus spp.	7 (5.4%)	0 (0.0%)	9 (7.2%)	0 (0.0%)	3 (3.9%)	0 (0.0%)	19 (3.6%)
Bacillus spp.	8 (6.1%)	0 (0.0%)	32 (25.6%)	14 (23.0%)	18 (23.4%)	12 (20.0%)	84 (16.1%)
M. haemolytica	33 (25.2%)	43 (60.6%)	14 (11.2%)	33 (54.1%)	1 (1.3%)	15 (25.0%)	139 (26.6%)
P. multocida	0 (0.0%)	0(0.0%)	3 (2.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (0.6%)
B. trehalosi	7 (5.4%)	14 (19.7%)	0 (0.0%)	0 (0.0%)	6 (7.8%)	5 (8.3%)	32 (6.1%)
Enterococcus spp.	1 (0.8%)	0(0.0%)	1 (0.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (0.4%)
Pseudomonas spp.	1(0.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)
Enterobacter spp.	5 (3.8%)	9 (12.7%)	6 (4.8%)	7 (11.5%)	2 (2.6%)	10 (16.7%)	39 (7.5%)
Klebsiella pneumoniae	3 (2.3%)	3 (4.2%)	0 (0.0%)	1 (1.6%)	1 (1.3%)	0 (0.0%)	8 (1.5%)
Actinobacillus ligneresii	3 (2.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	6 (7.8%)	2 (3.3%)	11 (2.1%)
Arcanobacterium pyogens	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (2.6%)	0 (0.0%)	2 (0.4%)
Unidentified	9 (6.9%)	0 (0.0%)	13 (10.4%)	0 (0.0%)	2 (2.6%)	0 (0.0%)	22 (4.2%)
Total	131	71	125	61	77	60	522

Isolation Rate of Bacteria from Lung and Trachea of Ruminants from Anaerobic Cultures: A total of 522 bacterial isolates were detected on blood agar plates cultured anaerobically; out of these 199 (38.12%), 186 (35.63%) and 137 (26.25%) were recovered from sheep, goats and cattle, respectively.

Fifty-two (39.7%) of the isolates observed from sheep lung were *Staphylococcus* species; 33 (25.2%) were *M. haemolytica*, 8 (6.1%) were *Bacillus* species, while *B. trehalosi* and *Enterococcus* species constituted 7 (5.3%) each from the total isolates and taken as a whole 10 bacterial species were recognized. Whereas, 43 (60.6%) *M. haemolytica*, 14 (19.7%), *B. trehalosi* and 9 (12.7) *Enterobacter* species were the majorly isolated species among the 5 isolated bacterial species which contributed for a total of 71 isolates of anaerobic sheep trachea culture (Table 2).

Among those bacteria cultured from goat lung tissue anaerobically 8 species of bacteria were determined and the majority of them were taken by *Staphylococcus* species which was 45 (36%) followed by *Bacillus* species, 32 (25.6%) and *M. haemolytica* 14 (11.2%) out of the total 125 isolates. But in the case of tracheal culture only 5 species were recorded and of which *M. haemolytica* accounts for 33 (54.1%) followed by *Bacillus* species, 14 (23%) and *Enterobacter* species, 7 (11.4%) from the total of 61 isolates.

Eleven different species of bacteria were known from anaerobic culture of lung of cattle. The most frequently isolated species were *Staphylococcus* 33 (42.9%), *Bacillus* species 18 (23.4%) and *B. trehalosi* 6 (7.8%), while the most prevalent species in the trachea of cattle were *M. haemolytica* and *Staphylococcus* 15 (25.0%) each followed by *Bacillus* species 12 (20.0%) and *Enterobacter* species 10 (16.7%) from the total of 7 identified bacterial species. The other species isolated and identified from pneumonic cattle lung and their respective trachea is shown in Table 2.

There was statistically significant difference ($p<0.05$) between anatomical site and species of animal in terms of the species of bacteria.

DISCUSSION

Respiratory tract infections are of a common occurrence in ruminant species of animals. It is considered that the bacterial flora of the respiratory system includes both resident and transitory microflora [18]. The causes of pneumonia in ruminants reflect a mixture of inter-related variables and stress factors that culminate in bacterial species [19].

This study has shown that a wide diversity of bacterial flora colonizes the respiratory passageways of pneumonic ruminants. This is supported by several workers who previously isolated different bacterial species from pneumonic lungs of sheep [20-23] and also from pneumonic caprine lungs [23-26]. In addition, Shemsedin [27] isolated multiple bacteria from pneumonic lungs of camels. The invariable isolation of these organisms from the pneumonic lungs of various animal species might indicate their role in causing different respiratory syndromes especially when the immune system of the animals is compromised by some other external factor.

In this investigation the isolation and identification of multiple bacterial species from the respiratory tract of ruminants were achieved from abattoir survey. Even though, the proportion of *M. haemolytica* vary from one species of animal to the other and as well as from each anatomical site, the present study discovered that the isolation of this bacteria is relatively in higher proportion in the tracheal culture of sheep when compared to the lung tissue culture. But the rate of isolation of this species was relatively decreased when it is isolated from the trachea and lung of goats. It is also considered that the rate of isolation of this species of bacteria is even less in cattle trachea and in the lungs of this species of animal. This agrees with the studies of multiple workers who describe *M. haemolytica* as a normal inhabitant of upper respiratory tract as well as a causative agent of secondary bronchopneumonia after primary initiating agent has suppressed the host defense mechanism [28, 29].

The results of *M. haemolytica* obtained from lung and trachea of goat in this investigation are higher than the results of Megra and his coworkers [26] which showed an isolation rate of 6% and 14% for trachea and lung of goats on their study carried out at Dire Dawa abattoir. But on the other hand, the result is lower than earlier reports of 35.5% by Almeida and his colleagues [24], 47.5% by Mohammed [25] and 18.8% by Tilaye [23] of lung infection rates. This variation might be attributed to the difference in agro-ecological zones of the study areas and also the health status of the respective animal species.

The isolation of *M. haemolytica* from pneumonic lungs of sheep at a higher rate agrees with the reports of Tesfaye [30] and Aschalew [31] who reported an isolation rate of 67.6 and 63.8% respectively. But much higher than the report made by Tilaye [23] is reported who presents an isolation rate of only 28.4%. The rate of isolation of these bacteria is higher except those isolated from cattle lung (1.3%) when incubated with increment of CO_2 tension.

The results of the abattoir survey also showed that the percentage of isolation of *B. trehalosi* from the trachea and lungs of cattle is higher when compared to the isolation rate from small ruminants. The isolation rate from the pneumonic sheep lung was closely related with the reports of Tilaye [23] who demonstrated a rate of 6.6%. In the present study *B. trehalosi* has not been isolated from either trachea or lung of goats. Unlike that of *M. haemolytica* anaerobic incubation does not improve the growth of *B. trehalosi* except in the case of cattle lung and this result was in agreement with the study of Sisay and Zerihun [32] who presented isolation of *B. trehalosi* at 67% rate from nasal swabs collected from the highlands of Wollo. The decreased isolation rate of *B. trehalosi* in this study when compared to the previous works might be that these authors isolated the bacteria from tonsils in addition to lung and nasal swabs and *B. trehalosi* is generally isolated at higher rate from tonsils than from lung.

The current investigation also indicated that the isolation rate of *P. multocida* in all the three species was very low. Except for the lungs of goats the bacteria was not isolated from the rest sampled animal tissues. The isolation rate has increased when the lung tissue was cultured anaerobically revealing its presence at 2.4% rate in goat lung. This relatively agrees with Tilaye [23] who isolated this bacterium from pneumonic lungs of goats and presents a 5.4% rate and failed to isolate the bacteria from pneumonic lungs of sheep. This is in accordance with previous reports [24, 25], indicating that the organism lives as a commensal in the upper respiratory tract and invading the lung under conditions of stress [33].

In general, the highest frequency of isolation of *M. haemolytica* in comparison with *B. trehalosi* and *P. multocida* in this study coincided with the works reported by other workers. Most of the studies carried out on pneumonic lungs of sheep in Ethiopia revealed that *M. haemolytica* has higher rate of isolation than *P. multocida* and *B. trehalosi* [23, 30, 31] and thus, has a much higher contribution in the occurrence of RDC.

The isolation of *Bordetella* species from the lungs of the three species of animals was the significance of this study. Even though, the isolation rate from sheep and goats is lower in comparison with cattle, its presence in the respiratory tract showed the importance of this bacterium for the contribution of RDC. Even if, previous studies in our country did not indicate the significance of these bacteria, Ngom together with his colleagues [34] reported an isolation rate of 3.3 and 4.8% from sheep and goats, respectively in their abattoir surveillance and indicate that the importance of *B. bronchiseptica* as a RDC is increasing from time to time in Senegal. The failure to isolate this bacterial species in previous reports from the country may be due to giving little consideration for possible role of the organism in respiratory tract health and disease so that they could not characterized suspected colonies.

The isolation of high proportion of other bacterial species from the lung and trachea of ruminants in general in the study signifies that a numbers of factors are involved for the contribution of respiratory infection in these animals. The findings of this study are in agreement with the previous study conducted. Yimer and Asseged

[22] isolated *E. coli, Staphylococcus, Streptococcus, Pseudomonas, A. pyogens, Enterococcus, Micrococcus, Enterobacter, Citrobacter* and *Klebsiella* species with different proportion from the nasal cavity, tonsil, trachea and lung of sheep in addition to major pathogenic species. While Megra and his co-workers [26] reported the presence of these bacteria in the respiratory tracts of goats. But the major difference observed from these studies was the presence of higher proportion of *E. coli* and *Corynebacterium* species in the above mentioned studies, which were not reported in the current study. Even though these groups of bacteria have not been considered as a major causative agent of RDC, their significance should not be disregarded.

Given the complex etiology of respiratory disease attributed to a particular species of bacteria, it is unlikely that any single strategy will be completely effective in preventing the disease. A combination of more definitive diagnostic methods, more efficacious vaccines, improved therapeutic agents and more rational management practices should be considered for preventing this highly devastating disease. It is evident from this study and previous works that respiratory infection is a highly complex multifactorial disease of a worldwide prevalence and distribution in cattle, sheep and goats. The disease primarily results from interaction of stress, immunity and the causative bacteria which is commensally resident in the respiratory tract of susceptible animals. The major factors leading to stress and compromised immunity are naturally created by adverse environmental and climatic conditions and also by previous or coinfection with certain respiratory viruses, *Mycoplasma* or some other types of bacteria.

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