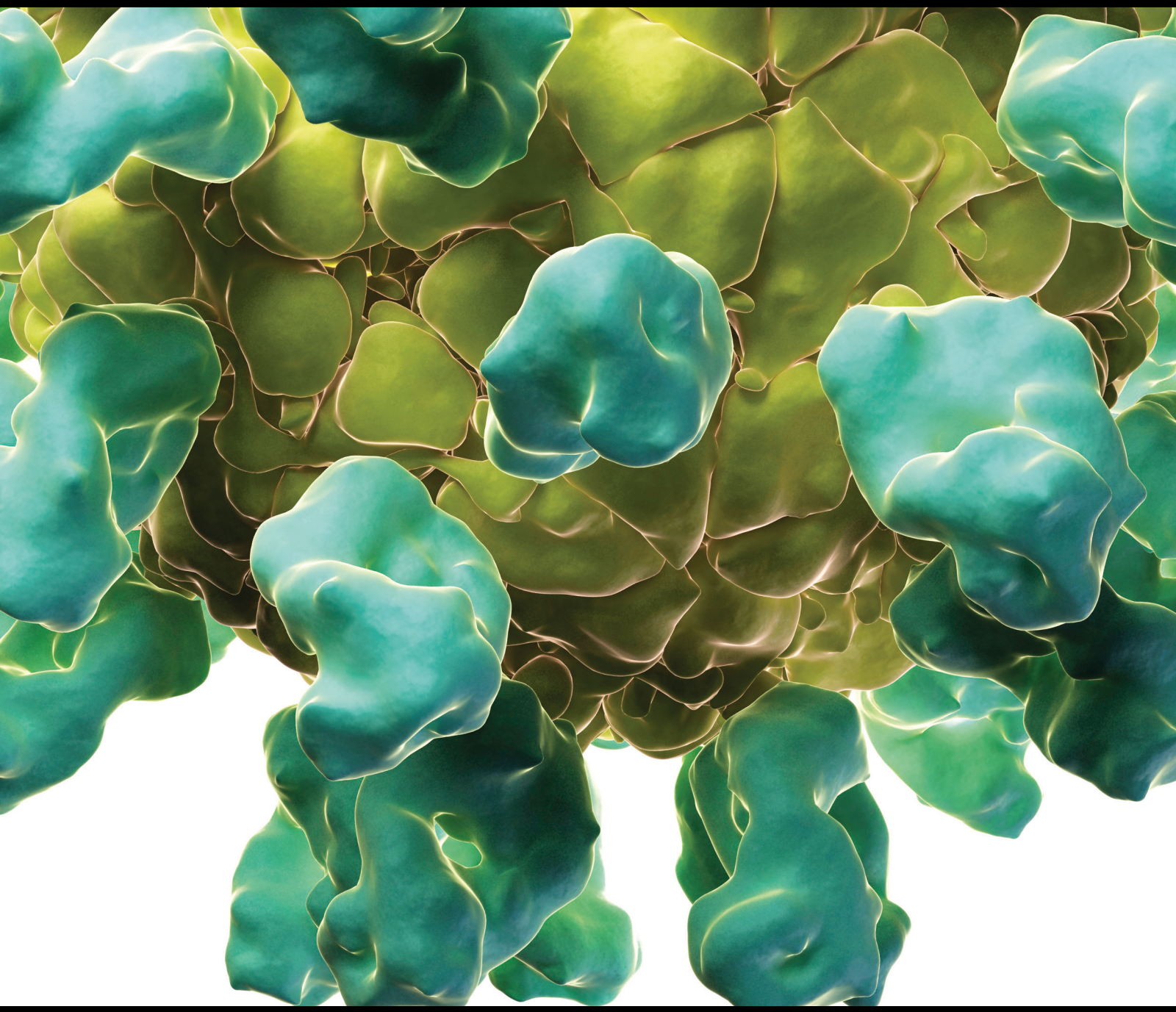
Veterinary Medicine International

Respiratory Diseases of Small Ruminants

Guest Editors: Amit Kumar, Suresh K. Tikoo, Praveen Malik, and Aruna T. Kumar



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Editorial Board

Contents

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Editorial

Respiratory Diseases of Small Ruminants

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Small ruminants are valuable assets for the Mediterranean, African, and Southeast Asian countries with the potential for providing meat, milk, and wool. These animals are highly susceptible to respiratory diseases, which account for almost 50% mortality amongst them. Irrespective of the etiology, the infectious respiratory diseases of sheep and goats contribute to 5.6 percent of the total diseases of small ruminants. The infectious respiratory disorders are classified into two groups: the diseases of upper respiratory tract including sinusitis caused by the larvae of parasites, nasal foreign bodies, gaseous irritation, and enzootic nasal tumors and the diseases of lower respiratory tract comprising mainly pneumonia. Often these are of infectious origin (bacterial, viral, or fungal). However, the role of the environmental pollutants, toxicants, and mechanical induction of respiratory distress may also be the cause of these abnormal conditions. Depending upon the environmental, physiological, and etiological factors, respiratory conditions might be acute, chronic, and/or progressive in nature.

To overcome such important disease conditions, information on their identification, prevention, cure, and control can improve the economic status and sustainability of holders of small ruminants. Thus an early, rapid, and specific diagnosis of such diseases holds great importance to reduce the losses. The advanced enzyme-linked immunosorbent assays (ELISAs) for the detection of antigens as well as antibodies directly from the samples are primarily available for all the disease conditions with specificity and sensitivity. Similarly, molecular diagnostic assays along with microsatellites comprehensively assist in diagnosis as well as treatment and epidemiological studies. The two reviews written by Chakraborty et al. and Kumar et al. discuss the advancements made in the diagnosis of common infectious respiratory diseases of sheep and goats, in general, and also the management of *Mycoplasma agalactiae*, a causal agent of classical contagious agalactia (CA). CA is a serious, economically important but neglected enzootic disease of small ruminants. Both reviews would certainly assist in designing appropriate prevention protocols and devising suitable control strategies to overcome such important respiratory diseases, thus alleviating the economic losses. The cross-reactivity and differential immunogenicity between *Mycoplasma agalactiae* and *Mycoplasma bovis*, two often confusing pathogens causing contagious agalactia, have also been elaborated in the research article authored by Kumar et al.

The research article by P. Scott details the antibiotic treatment response in sheep with chronic lung diseases where the diagnosis was based upon ultrasonographic examination of the lungs using 5 MHz linear and sector scanners with no consideration to the auscultation findings. The lung pathological examinations were considered a basis for comparison between sonographic findings and pathological changes.

Rahal et al. described in detail how a number of pathogenic microorganisms have been implicated in the development of respiratory diseases but the importance of environmental factors in the initiation and progress of the disease can never be overlooked. These environmental factors irritate the respiratory track producing stress in the microenvironment causing a decline in the immune status

of the small ruminants and thereby assisting bacterial, viral, and parasitic infections in breaking down the tissue defense barriers. Environmental pollutants cause acute or chronic reactions as they deposit on the alveolar surface, which are characterized by inflammation or fibrosis and the exhibition of transitory or persistent tissue manifestation.

The disease development can be portrayed as three sets of two-way communications among pathogen, environment, and host but the interactions are highly variable. Moreover, the environmental scenario is never static; new compounds are introduced daily making a precise evaluation of the disease burden almost impossible. The last article in the issue presents a detailed overview of these interactions and the ultimate effect on the respiratory health of sheep and goat.

Acknowledgments

The editors thank the authors for their efforts and time spent for each paper. The lead editor thanks all editors for the time spent in reviewing, assigning reviews, and commenting on submitted papers. The editors hope that this special issue will prove useful to investigators, policy makers, and veterinarians involved in the study of respiratory diseases of small ruminants. Last but not least, the expertise and cooperation of editorial team are highly acknowledged.

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Review Article

Mycoplasma agalactiae, an Etiological Agent of Contagious Agalactia in Small Ruminants: A Review

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Mycoplasma agalactiae is one of the causal agents of classical contagious agalactia (CA), a serious, economically important but neglected enzootic disease of small ruminants. It occurs in many parts of the world and most notably in the Mediterranean Basin. Following the infection common complications are septicaemia, mastitis, arthritis, pleurisy, pneumonia, and keratoconjunctivitis. Primary or tentative diagnosis of the organism is based upon clinical signs. Various serological tests, namely, growth precipitation, immunofluorescence, complement fixation test, haemagglutination inhibition, agglutination, immunodiffusion, enzyme immunoassays, immunoelectrophoresis, blotting techniques, and others, are available. Molecular tools seem to be much more sensitive, specific, and faster and help to differentiate various strains. The real-time PCR, multiplex PCR, quantitative PCR, PCR-RFLP, MLST, and gene probes, complementary to segments of chromosomal DNA or 16S ribosomal RNA (rRNA), have strengthened the diagnosis of *M. agalactiae*. Both live attenuated and adjuvant (alum precipitated or saponified) inactivated vaccines are available with greater use of inactivated ones due to lack of side effects. The present review discusses the etiology, epidemiology, pathogenesis, and clinical signs of contagious agalactia in small ruminants along with trends and advances in its diagnosis, treatment, vaccination, prevention, and control strategies that will help in countering this disease.

1. Introduction

Contagious agalactia, a disease with the involvement of multiple organs, produces systemic infections and is supposed to be among the most serious diseases of small ruminants, produced by mycoplasmas after contagious caprine pleuropneumonia (CCPP) [1–3]. In many parts of the world countries most notably in the Mediterranean basin, are severely affected economically due to outbreaks. It is a listed disease by World Organization for Animal Health (OIE), which is responsible for severe losses to dairy industry [4, 5]. *Mycoplasma agalactiae* is the classical etiological agent of this disease which primarily affects goats and sheep along with many wild species. The impressive diffusion of this disease is due to several factors including primitive herding practices, inefficiency of antimicrobial therapies, and adoption of very few prophylactic measures [6]. The disease has been reported from almost all the countries and continents of the world and is responsible for heavy economic losses to shepherds mainly due to high morbidity rather than high mortality in sheep population throughout the world [1, 4, 7–9]. In the European countries major economic losses are incurred upon by the disease due to reduced or suppressed production of milk and abortion along with high morbidity as well as mortality rates

in adult sheep. Along with this the cost of diagnosis is a major problem which has been estimated to be approximately 20 million Euros for a year [3, 10].

Mycoplasma agalactiae is the second one in mycoplasma species, after *M. mycoides* subsp. *mycoides* type SC. It was first reported, dating back to 1923, when Bridre and Donatien cultivated the microbe responsible for causing contagious agalactia (CA) in goats for the first time [11]. In 1925, Bridre and Donatien for the first time reported CA as a disease of sheep and goats characterized by mastitis, arthritis, and keratoconjunctivitis and succeeded in growing the causal organism [12]. However, the disease was first notified in 1816 in Italy. Initially in 1931, the organism was named as *Anulomyces agalaxie* [13] and after the advent of new taxonomy of mycoplasmas, Freundt named it *Mycoplasma agalactiae* [14]. Initially, *M. agalactiae* was considered to be the classical etiological agent of contagious agalactia [4]. However, now this designation of *M. agalactiae* disease as "contagious agalactia" appears to be misnomer as disease occurs in both sexes. Further the involvement of other species is also well established in mycoplasma induced agalactia. It is because the complex of disease conditions, namely, mastitis, agalactia, keratoconjunctivitis, and pneumonia (MAKePS syndrome), which was earlier assigned to *M. agalactiae* is supposed to be due to the cluster including *M. mycoides* subsp. *mycoides* large colony type (LC), *M. capricolum* subsp. *capricolum*, and *M. mycoides* subsp. *capri* [15]. Moreover, a disease with almost similar clinical and pathological manifestations is also caused by *Mycoplasma putrefaciens* in goats [16]. Still *M. agalactiae* is supposed to be the major pathogen which accounts for 90% outbreaks of contagious agalactia syndrome in goats [17] and almost 100% in sheep [18, 19]. Most importantly, control and eradication of contagious agalactia can be obtained through better diagnostic tests and through a more efficient vaccine [20]. The present review discusses some salient features of *M. agalactiae* and the disease (contagious agalactia) caused in small ruminants with regards to epidemiology, pathogenesis, and clinical signs, along with focusing the trends and advances on its diagnosis, treatment, vaccination, prevention, and control strategies that will help in countering this disease in a better way.

2. Etiology

2.1. Morphology, Cultural and Biochemical Characteristics. *Mycoplasma agalactiae* is a polymorphic bacterium with the size in the range of 124–250 nm and has a very small genome (1×10^9 Da). The isolation of *M. agalactiae* is bit time taking due to slow adaptation of bacterium to new environment. Freshly isolated strains of the bacterium are slow growing, but when adapted to laboratory conditions these grow easily in majority of the commonly used media for mycoplasma growth [21, 22]. *M. agalactiae* produces colonies with dark centers producing typical fried-egg appearance and this phenomenon is called as "film and spot". In biochemical characterization, *M. agalactiae* neither ferments glucose nor hydrolyses urea and arginine [18, 23, 24]. Staining of the mycoplasma colonies is performed with Giemsa stain from solid agar media to observe the colony characteristics [21, 22]. The absence of cell wall in the mycoplasma leads to pink color staining with Gram staining [21, 25].

2.2. Growth Requirements. Initially it takes few days to a week time to grow *M. agalactiae* in laboratory media, but the growth time is reduced after adaptation [22, 26]. Moreover, the growth of strains is comparatively slow in solid media in comparison to liquid media [22]. *M. agalactiae* is routinely grown at 37°C in laboratory media enriched with sterol [22, 27]. The growth on solid media in humid atmosphere is supported by 5% CO_2 and the osmotic pressure of 7 to 14 atmospheres [7, 22]. *M. agalactiae* multiplies by budding or binary division and grows well on special liquid and solid media with the addition of sterols, which is an essential component for the synthesis of plasma membrane. As the organism is sensitive to alteration in pH, optimum pH should be maintained at 7.6 with the addition of organic components like DNA and NADH to improve the growth [21, 22, 28].

2.3. Sensitivity and Resistance. *M. agalactiae* is very sensitive to high temperature and can be easily inactivated with the exposure to 60°C for 5 min. and within a minute at 100°C. It can be inactivated with the direct exposure to sunlight during hot summer season. The survival time of the organisms varies from 1-2 weeks to 3-4 months at room temperature and in refrigerator at 8°C, respectively, depending upon other conditions like pH of media. Humid and cold conditions support its survival. It can survive for 8 to 9 months of period at −20°C. Exposure to ultraviolet radiation and dyes inactivates it quickly. Moreover, the organism can be easily destroyed by commonly used disinfectants such as potassium hydrochloride, formalin, and chloramines [16]. Similar to other mycoplasma species *M. agalactiae* also lacks cell wall and, due to the presence of only the plasma membrane, it is resistant to penicillin and its analogues. However, its cells are sensitive to digitonin. The presence of only plasma membrane makes it vulnerable to osmotic shock and the effect of detergents [21, 29].

2.4. Antigenicity. In 1968, Razin [30] applied polyacrylamide gel electrophoresis (PAGE) to study the electrophoretic patterns of mycoplasma cell proteins to resolve several taxonomic problems in the Mycoplasmatales. He observed the similar patterns for several mycoplasmas, namely, *M. mycoides* subsp. *capri*, other caprine mycoplasmas, *M. agalactiae* and *M. agalactiae* var. *bovis*, and different murine mycoplasmas. However, the avian mycoplasma species, namely, *M. gallisepticum*, *M. synoviae*, *M. meleagridis*, *M. gallinarum*, and *M. iners*, showed easily distinguishable and specific patterns. *M. agalactiae* has many cross-reactive antigens of heterogenous nature; hence, initially due to lack of knowledge regarding its protein heterogeneity, it was reported to be a species with uniform antigenicity [31, 32]. *M. agalactiae* and *M. bovis* are almost identical in cell and colony form as well as in their metabolic behavior with the sharing of high number of antigens. It is difficult to differentiate them on the basis of usual morphological, metabolical, and

serological methods [21, 33–35]. Now the antigenic heterogeneity of *M. agalactiae* has been duly established [22, 36–41]. In a recent study, SDS-PAGE revealed 24 polypeptides in whole cell antigens (WCA) and sonicated supernatant antigen (SSA) of Indian isolates of *M. agalactiae*, respectively. They are in the range of 20.89 to 181.97 kDa with seven major proteins of 63.10, 60.25, 58.88, 47.86, 44.66, 33.88, and 28.84 kDa molecular weights. On immunoblotting with polyclonal rabbit serum produced against *M. agalactiae*, all the major proteins appeared immunogenic with 12 to 14 immunogenic polypeptides [42]. These major immunogenic proteins are being targeted for the development of diagnostic aids for the detection as well as differentiation of *M. agalactiae* from other related mycoplasmas.

3. Epidemiology

The disease primarily occurs in Mediterranean countries [43, 44]. *M. agalactiae* has been reported to be isolated from different parts of the world in various countries, namely, India [45], Australia [46], Turkey [47], Iran [48], Mongolia [49], Nigeria [50], Senegal [51], Iraq [52], and Spain [3]. Apart from the above it has also been reported from regions and countries such as European litoral, Bulgaria, Serbia, Sudan, Russia, Asia Minor, America, and Switzerland [4, 18, 53]. Thus by the end of the 19th century the disease had become enzootic in many parts of the world [41].

The disease has also been noted in the countries of West Asia; Central as well as North and East Africa; the United States as well as Brazil. In both sheep and goat population of Jordan, *M. agalactiae* is the major pathogen causing the disease contagious agalactia. In the Western Pyrenees basin of France there has been reemergence of this particular pathogen. There is however research gap regarding the epidemiology of the disease in Spain which is among one of the countries of European Union containing large population of sheep [54, 55].

M. agalactiae infection represents a risk for population density and maintenance in wild populations, namely, *Iberian ibex* (*Capra pyrenaica*) in Spain [56]. The predisposing factors for the occurrence of the disease are sex (in females), age (young animals), and metapopulation [57]. In the population of wild ibex the strains of *M. agalactiae* have been found to be highly related and appeared to originate from an individual parental clone spreading to another species of wild ungulate (chamois) in same geographical location. Strains found in Europe are clearly different from those found nearby. The pathogenesis of *M. agalactiae* infection is not clear in ibexes, but in Alpine there has been atypical strain emergence. This has given rise to the thought that wild fauna can act potentially as reservoirs of mycoplasmas that are pathogenic [5].

4. Transmission of Disease

The sustainability of organism at room temperature supports its rapid spread through contact from infected to healthy animals. The main sources of infection include auricular, ocular and nasal secretions, faeces, milk, urine, and excretions from joint lesions [58]. Sexual transmission through infected male has been reported. Contaminated utensils and milker's hands are vital source of infection. Vertical transmission is observed through contaminated colostrum or milk [18, 59, 60]. The various sources of disease transmission have been depicted in Figure 1.

In majority of cases chronic or persistence infection for several months in flock is observed with clinically positive animals during favorable environmental conditions as at the time of hot and humid summer. Young, malnourished, pregnant, and immunocompromised animals are comparatively more susceptible to the infection [61]. There are reports of excretion of organisms in milk even after 8 years of infection with mild and with or without clinical signs [37, 62]. Thus the presence of asymptomatic carriers in a herd which carry the infectious agent is of major concern. Persistence of antibodies could be observed up to 8 and 3 years of clinical disease in goats and sheep, respectively [63, 64]. Animal species other than homologous hosts as cattle, camel, and many other wild small ruminants can also act as reservoir of the infection. These carrier states are more frequently observed in females, particularly in their genital tracts [16, 56, 65].

5. Pathogenesis

M. agalactiae is comparatively stable at room temperature and in general is transmitted through oral, respiratory, and mammary route. The different routes of transmission and process of disease development have been depicted in Figure 1. It has been isolated from nasal secretions [21, 66, 67], faecal samples [68], milk [59, 69], and aborted fetus [70]. It suggests that the primary site of predilection is the mucosa of respiratory tract, small intestine, and alveoli of mammary glands, respectively, depending upon the respiratory, oral, and mammary routes [21]. However, as such no disease condition is reported with the involvement of small intestine. Once infection is set up, fever is observed due to bacteremia accompanied by fever. Then following the initial multiplication organisms are disseminated through circulation to different vital organs, namely, lungs, lymph nodes, eyes, mammary glands, joints, and tendons, producing various clinical signs [8, 26]. Involvement of connective tissues in mammary glands leads to initial inflammation which ultimately turns in catarrhal or parenchymatous mastitis leading to atrophy and agalactia [21, 53]. Animals suffering from mastitis can spread disease to young ones through colostrums or milk [71]. In general, lung lesions are observed with *M. agalactiae* infection, although outbreaks of pleurisy among goats with the isolation of mycoplasma have been reported [8, 43]. Painful swelling of joints with the accumulation of synovial fluids leads to arthritis mainly in carpal and tarsal joints. In chronic cases eventual loss of joints leads to ankylosis. Affections in eye cause severe losses of cornea, ultimately leading to blindness through vascularisation and keratoconjunctivitis [26, 60, 72, 73]. Affections of genital organs are also observed with occasional abortions or still births in pregnant animals, mainly due to the inflammation

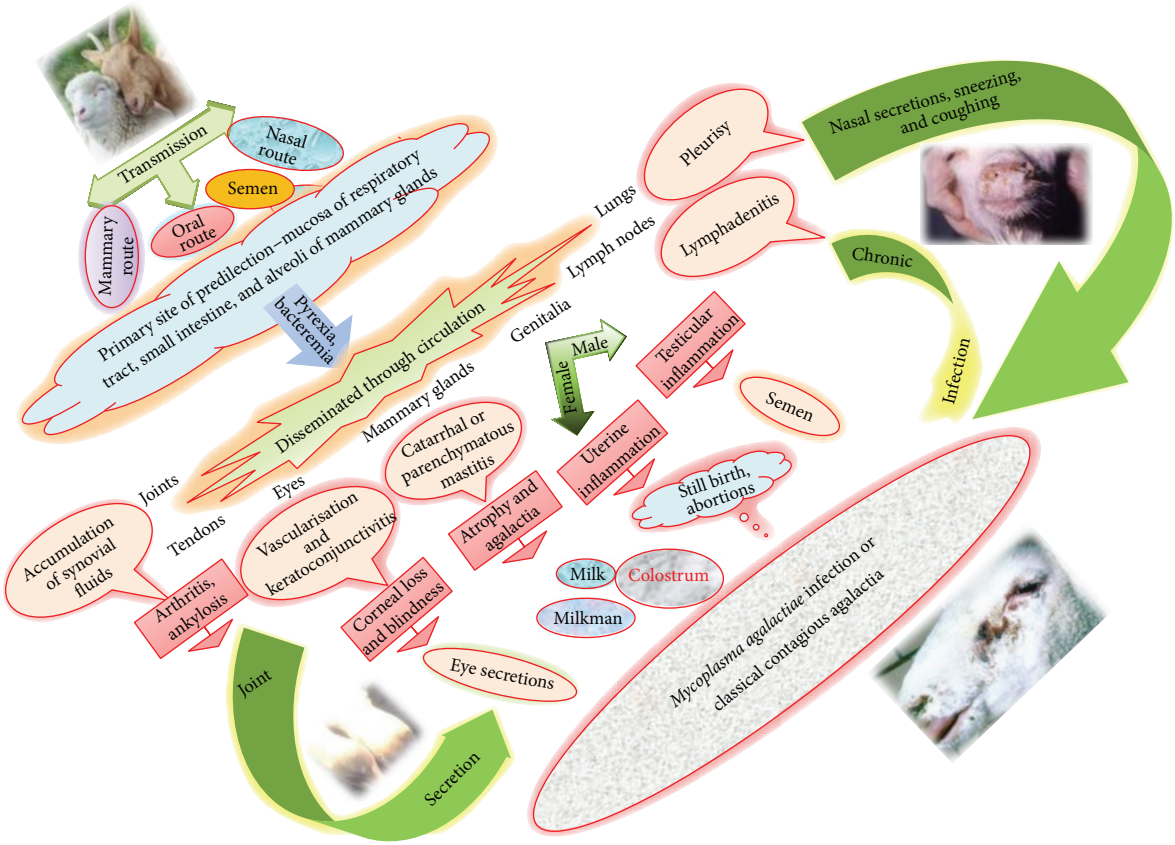

Figure 1: Transmission and pathogenesis of *Mycoplasma agalactiae*.

of the uterus. In male animals it may produce testicular inflammation. The association of *M. agalactiae* with granular vulvovaginitis in goats has been observed [8, 74]. Despite all kinds of clinical affection and metabolic alteration, *M. agalactiae* infection in goats does not produce anaemia or septicaemia [26]. However, the presence of mycoplasma in circulation, that is, mycoplasmamia, is mainly responsible for its dissemination in various organs, particularly in sheep. The disease conditions produced by *M. agalactiae* are responsible for high economic losses which are due to the loss of milk and loss of lambs and kids because of abortions, neonatal deaths, and loss of animals themselves. Moreover, losses also occur due to the losses caused by subacute, acute, and chronic forms of disease in the form of physical weaknesses and the clinical complications which affects the animals in infected herd [35, 50, 74, 75].

5.1. Pathogenicity in Laboratory Animals. Pathogenicity of *M. agalactiae* in the laboratory is tested experimentally on mice [76]. The young mice are inoculated through intraperitoneal route with 24 hours of grown young cultures. Then after 24, 48, and 96 hours, the tail blood is applied in liquid and solid media. To judge the presence of mycoplasmaemia in liquid and on solid media, color change due to pH alteration in liquid media and presence of fried-egg colonies are observed on solid media [8, 76–78].

6. Clinical Signs

M. agalactiae can affect both sheep and goats of either sex. The incubation period of the organism varies from few days to few weeks and even up to two months depending upon the route of entry, number and virulence of organisms, and immune status of the animal [8]. Young animals which are deprived of maternal antibodies, weak, debilitated, malnourished, and immunocompromised and animals under stress during and after transportation, under physiological stress like pregnancy, and exposed to extreme climatic conditions are frequently affected. Depending upon such conditions *M. agalactiae* can produce acute, subacute, or chronic form of disease. In some animals atypical or asymptomatic forms have also been reported [36, 71, 79, 80]. Common clinical symptoms include fever, anorexia, lethargy, and unwillingness to follow the herd, followed by the clinical symptoms depending upon the involvement of various organs such as mammary glands, lungs, genitalia, joints, and conjunctiva. Rare abortions in pregnant animals have also been reported [21, 26, 81]. Importantly, fever is common in acute cases and may be accompanied by nervous signs, but both signs are rare in the more frequently observed subacute and chronic infections. *M. agalactiae* may occasionally be found in lung lesions [82]. However occurrence of pneumonia is not a consistent finding. Loss of milk production, discoloration, saltiness, and change of consistency of milk and ultimately

agalactia are commonly observed. Young ones receiving infected colostrums and milk might lead to septicaemia, arthritis, or pneumonia with high mortality of the kids [8, 71]. Chronic involvement of joints and severe losses to cornea lead to lameness along with inability to walk or stand and blindness, respectively [72, 73]. The conditions like pleurisy, arthritis, pneumonia, keratoconjunctivitis, and mastitis usually result from infection with *M. mycoides* too because this organismhas one of the widest geographical distributions and is found wherever contagious agalactia is reported [69]. Congenital polyarthritis has also been reported from goat kid [83, 84]. The clinical conditions produced during infection have been elicited in Figure 1.

7. Diagnosis

7.1. Conventional Diagnosis. Primary or tentative diagnosis of the organism is based upon clinical signs, namely, loss of milk production, mastitis, keratoconjunctivitis, and articular lesions. Discoloration of milk in yellowish-green color, ocular discharges, articular swellings, and lameness are suggestive of *M. agalactiae* infection. The clinical diagnosis is confirmed by isolation and identification of the organism in the laboratory [85]. Samples of milk, auricular, ocular, vaginal, or nasal discharges, articular exudates, blood, and urine are used for the diagnosis [21, 26, 86]. For the isolation purposes from infected tissues, samples are collected aseptically from the mammary glands, regional lymph nodes, pulmonary lesions, and articular exudates during postmortem examination [26]. Isolation of *M. agalactiae* from liver, kidney, and spleen could be performed during the phase of mycoplasma. Cultivation is carried out in liquid or on solid media which support mycoplasma growth [18, 21]. *M. agalactiae* produces fried-egg colonies. Characterization of isolates based on biochemical tests is not usually recommended [22, 87] due to morphology, growth, and metabolic similarity to some other mycoplasmas [28, 88]. Various methods of diagnosis have been depicted in Figure 2.

7.2. Serological Diagnosis. Serological tests of importance for detecting *M. agalactiae* include growth precipitation (GP), immunofluorescence (IF), complement fixation test (CFT), indirect haemagglutination (IHA), haemagglutination inhibition (HI), agglutination, latex agglutination test (LAT), double immunodiffusion (DID), single radial immunodiffusion (SRID), enzyme linked immunosorbent assay (ELISA), radio immunoassay (RIA), and immunoperoxidase (IP) [1, 9, 22, 87, 89–96]. They also include many electrophoretic techniques such as gel electrophoresis, immunoelectrophoresis (IEP), countercurrent immunoelectrophoresis (CCE), and crossed immunoelectrophoresis [22, 40, 97, 98]. Immunoblotting has been used to demonstrate the antigenic specificity by the use of hyperimmune sera from rabbit which is monospecific [42]. Other than these methods, the techniques to separate protein antigens, namely, polyacrylamide gel electrophoresis (PAGE), sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), two-dimensional immunoelectrophoresis, western blotting, dot blotting, and immunobinding assay, have also been developed and attempted to diagnose caprine agalactia [34, 35, 40, 99–103]. However, to overcome the difficulties and limitations in identification of the organism, diagnosis of *M. agalactiae* can be carried out by the complement fixation test (CFT) or monoclonal antibody based ELISA techniques against individual mycoplasma species or by means of gene amplification techniques [36, 79, 80, 104]. Serological tests have been efficiently used for the diagnosis of contagious agalactia due to *M. agalactiae* from the field cases, but dependency of these tests on crude antigens, in general, may not render them very specific and sensitive. Therefore, many of these tests cannot differentiate between the mycoplasma species due to the presence of common antigens [22, 34, 85, 87]. For differentiation of *M. agalactiae* and *M. mycoides* (large colony) and many other related species monoclonal antibody as well as recombinant protein based ELISA has been described [36, 79, 80, 104]. In small ruminants, affected with contagious agalactia, correlation study conducted on ELISA activity with various other serological tests under the field conditions indicated the ability of the test to detect the subclinical infection caused by the organism and also the ability to screen the goat herds for the presence of carrier animals [9, 61]. The ELISA, CFT, and immunoblotting are supposed to be standard serological tests as per the guidelines of OIE.

7.3. Molecular Diagnosis. The recent advances in molecular biology and biotechnology have strengthened the diagnosis, characterization, and differentiation of mycoplasmas including *M. agalactiae*. Cross-reactive closely related species can be certainly differentiated by the use of gene probes, complementary to segments of chromosomal DNA or 16S ribosomal RNA (rRNA) [63, 105, 106] with mixed success (Figure 2). However, the use of polymerase chain reaction (PCR) technique that seems to be even more sensitive and effective tool for the identification purposes is commonly practiced [107]. A simple method for detection of *M. agalactiae* from sheep milk by DNA extraction and subsequent PCR has proven to be faster than cultural isolation of the organism and has reduced the time required for diagnosis from days to hours [39, 108–110]. Use of pulsed-field gel electrophoresis (PFGE) has also strengthened the *M. agalactiae* diagnostics [39, 111]. PCR techniques based on 16S rRNA [79, 112], uvrC gene [113], and multiplex PCR [15, 26, 100, 114, 115] are being routinely used for the identification of *M. agalactiae* and have high diagnostic value (Figure 2). Molecular detection based on uvrC gene is of prime importance according to the recommendation of OIE.

By amplifying the 16S rRNA gene it is possible to identify the *M. agalactiae* isolates by means of PCR and it has been found that 99.8 percent similarity is shared by *M. agalactiae* as well as *M. bovis* isolates. Certain other diagnostic strategies include unknown sequence amplification or amplification of certain particular gene apart from uvrC like mb-mp81 gene encoding the P81 membrane protein. PCR-restriction fragment length polymorphism (PCR-RFLP) technique forms the basis of this method [107, 116]. Real-time quantitative PCR

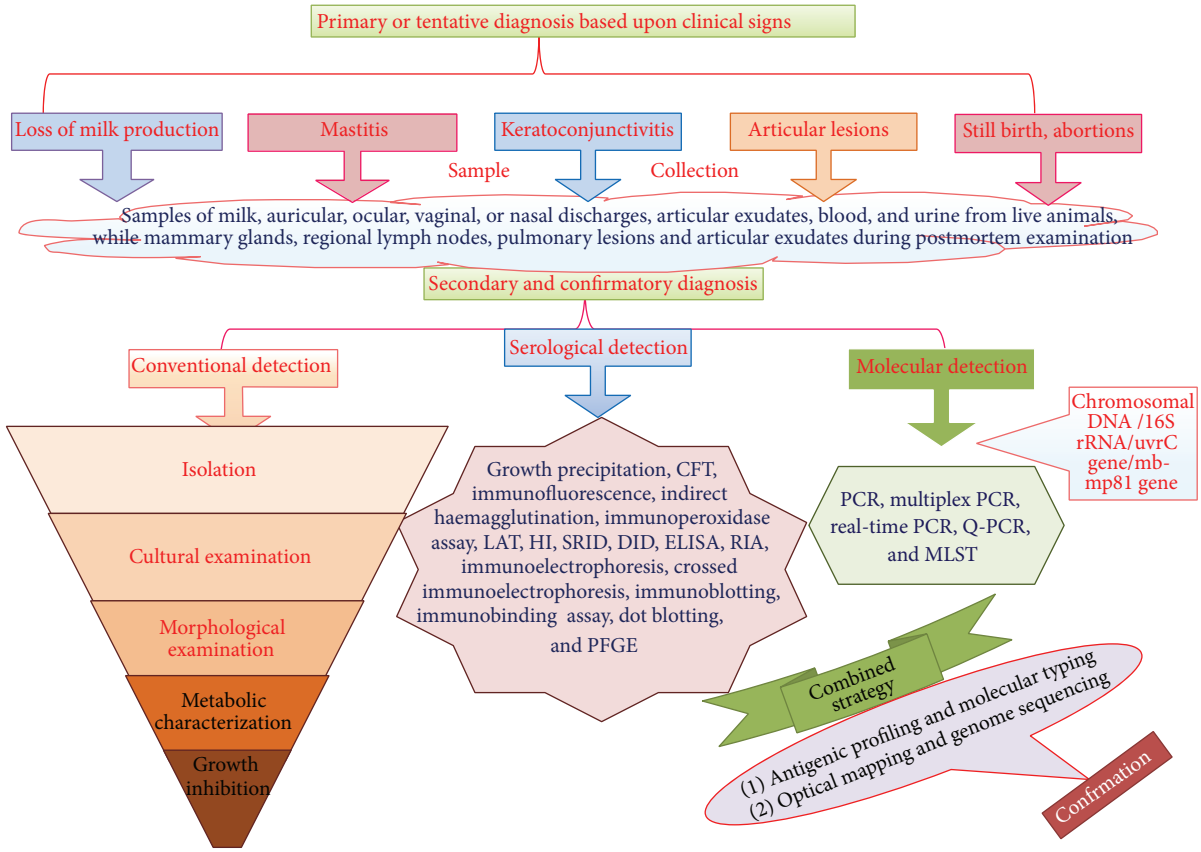

Figure 2: Diagnosis of *Mycoplasma agalactiae* infection.

(Q-PCR) assay has been used for quantifying the organism absolutely and is becoming increasingly popular for the purpose of diagnosis in both clinical and food microbiology [117]. Higher specificity as well as sensitivity of analysis is provided by this technique thus reducing the chances of cross-contamination. Chemistry of molecular beacon has been used by certain workers for developing a real-time PCR detection methodology. This method targets a region of 117 base pairs (bp) of the mb-mp81 gene of *M. agalactiae* encoding P81 lipoprotein gene [118]. In each of the reaction mixtures it is mandatory to add internal amplification control (IAC) for assessing the potential inhibitory effect of PCR or thermocycler malfunctioning. It is known that a chimeric nontarget DNA fragment is IAC present in each of the reaction mixtures and target sequence can be used to coamplify it [119]. However, in an another method, alternate template instead of being used in same PCR reaction mixture, it is run under similar amplification conditions in separate PCR wells [118, 120].

Multilocus sequence typing (MLST), a robust molecular tool, has also been used for comparison of genetic sequences of *M. agalactiae* [121, 122]. *M. agalactiae* possesses a capacity for phenotypic diversification of its surface antigens [36, 79]. In this regard, analysis of the antigenic variation of several *M. agalactiae* wild strains using different sera from naturally infected sheep followed by characterization of two strongly immunogenic membrane surface proteins of 55 kDa and 35 kDa, respectively [110] is quiet noteworthy. The gene encoding the P48 major surface lipoprotein has been characterized and reported to play a crucial role in the immune response of infected animals. Analysis of a recombinant P48 expressed in *E. coli* by using western blot and indirect ELISA proves to be a diagnostically relevant marker of *M. agalactiae* infection [123].

A combined strategy including antigenic profiling, molecular typing, and optical mapping as well as sequencing of the whole genome has shown the presence of 35 coding sequence. These sequences are based on gene involved, expression of antigens, and vice versa. They are contained in a large prophage and have confirmed the characterization of isolates in wild ungulates [5].

8. Treatment

Initial therapy for the infection included the use of arsenicals, particularly sodium and zinc salts of acetarsol. The use of these compounds and their continuous therapeutic usage had adverse effects. Presently all over the world preferred therapy is the use of antibiotics based on drug sensitivity. Commonly used antibiotics include tetracycline, macrolide, clindamycin, florfenicol, tylosin, tiamulin, tilmicosin, and fluoroquinolones [35, 124–126]. Systemic use of antibiotic responds well; however, local application in advanced stages

to avoid damages in mammary glands, conjunctiva, and joints should accompany systemic treatment [127, 128]. Fluoroquinolones, particularly enrofloxacin which is converted to ciprofloxacin after metabolism [129], might have less chances of resistance development. Moreover, the peak value of minimum inhibitory concentration (MIC) of ciprofloxacin is reached within few minutes in sheep [129–131] so it would be more useful in acute cases. The use of traditional antibiotics in acute cases is followed by the long acting preparations which have vital role in the subclinical and chronically affected animals. The confirmation and culling policy has limited the use of antibiotics; however precious animals and suspected animals are always treated with parental therapy followed by long acting oily preparations.

9. Prevention and Control

The multiple sources of infection and excretion of *Mycoplasma agalactiae* through various body secretions lead to rapid spread of infection. Thus the timely and quick response to the infection is essential for the prevention and control of the spread of infection to susceptible animals [35]. *M. agalactiae* infection could be prevented by adopting good managemental practices and following continuous surveillance/monitoring for the pathogen. Many times subclinically infected animals may also spread the infection; hence there is always a need to apply specific, sensitive and rapid diagnostic procedure for its early detection. Till confirmation suspected animals should be isolated and kept under observation. Immediately after disease confirmation, culling of all the contact and affected animals is recommended. Proper disposal of litter and other materials, namely, discharges and aborted fetus, and proper sterilization of contaminated utensils are recommended. Use of disinfectants as hypochloric acid, formalin, cresols, and phenolic substances along with commonly used quaternary ammonium compounds is effective against the organism [21, 35]. Proper screening of the semen for artificial insemination and bucks to be used should be conducted on regular basis. To avoid the vertical transmission ewes should be vaccinated. In endemic areas vaccination with locally developed vaccine is effectively applied throughout world.

10. Vaccines

Similar to many other bacterial agents, both live attenuated and inactivated vaccines are available for caprine agalactia [2, 111, 132–140]. These vaccines are both safe and effective [68, 137, 138, 141, 142]. The vaccination against *Mycoplasma agalactiae* in sheep induces both specific and nonspecific, humoral, and cellular response irrespective of type of vaccine. However, duration of persistence of antibodies depends upon multiple factors, namely, strain used, adjuvant incorporated, dose of vaccine, routes of inoculation, physiological status of animal, and so forth. Live attenuated vaccines are more effective and have been reported to provide better protection in ewes and their lambs than the inactivated vaccines but can produce a transient infection with shedding of mycoplasma through milk. Importantly, the live vaccines should be part of a regional plan in which all flocks from which animals are likely to come into contact be vaccinated at the same time. Inactivated vaccines are much safer with no side effects but have shorter period of protection with doubted efficacy [71, 140, 143]. It is possible that in some instances the apparent lack of protection given by vaccines can be the result of infection of animals with one of the other four mycoplasmas involved in the contagious agalactia syndrome. The issue for vaccine is duration, the levels of immunity are being addressed, and a combined inactivated vaccine with aluminium hydroxide gel and saponin and with mineral oil as adjuvant was also attempted in laboratory [139, 140] and field condition [137, 138, 144]. A saponified vaccine was reported to be effective in initial laboratory trial in mice [145]. Three inactivated vaccines, namely, A, B, and C, with several adjuvants (oil-emulsified) prepared with *M. agalactiae* have been evaluated for immunogenicity as well as efficacy purposes. For this purpose, animals have been divided into three groups and immunized with same vaccine using different adjuvant. After challenge with the organism, clinical protection has been induced by all the vaccine formulations. Full protection however has been induced by only the vaccine C which contains Montanide ISA-563 as well as Marcol-52 and Montane-80 (ratio: 30% : 63% : 7%) and has been found to induce protection at full level in animals that are challenged. This has helped in preventing both the clinical signs' onset and infection [136]. Out of available live attenuated and adjuvant (alum precipitated or saponified) vaccines, inactivated vaccines are of greater use due to lack of side effects. However, the protection period is short in comparison to live vaccines.

11. Conclusion and Future Perspectives

Contagious agalactia is considered as a neglected disease of small ruminants because of the complex disease distribution pattern, ubiquitous nature of the causal agent, and poor sheep and goat farm managemental practices, especially in developing and underdeveloped countries like India. Rapid spread and multiple sources of infection along with vertical and horizontal mode of transmission are matter of immense concern and severely affect the local economy. Depending upon conditions like deprivation of maternal antibodies, immunocompromised state, stress due to transportation, pregnancy, or extreme climatic conditions, animals may suffer from acute, subacute, chronic, or asymptomatic forms of disease. The isolation of *M. agalactiae* is a difficult task due to its property of lack of cell wall resembling other organisms of the genus *Mycoplasma*, and serological tests can efficiently identify *M. agalactiae*. However, in recent years, the isolation of *M. capricolum* subsp. *capricolum* (*Mcc*) and *M. mycoides* subsp. *capri* (formerly *M. mycoides* subsp. *mycoides*; large colonies) from sheep and goat having mastitis and arthritis complicated the situation. The cross-reactivity of many antigens of these mycoplasmas may lead to false reactivity. Under such conditions, the advanced molecular detection techniques like 16S rRNA based PCR and multiplex

PCR certainly help in differentiation of closely related species of the organism which often cause confusion in the mind of the diagnostician. Systemic uses of tetracycline, macrolide, or quinolone group of antibiotics along with local application in advanced stages are useful treatment options. Good managemental practices like isolation of sick animals along with test and slaughter policy form the basis of disease prevention. For the prevention and control of disease in particular to endemic areas vaccination is only effective strategy. Mono-, bi-, and trivalent live attenuated and adjuvant inactivated vaccines are available with local strains with limited success rate. However, further research on the molecular epidemiology of the organism in both domestic and wild animals is necessary to fully understand the disease distribution pattern to effectively manage the populations of goat and sheep and protect them against the infection. Similarly, there is need to explore the advancements made in the field of vaccinology for the management of the disease more efficiently in sheep and goat population.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Review Article

Advances in Diagnosis of Respiratory Diseases of Small Ruminants

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Irrespective of aetiology, infectious respiratory diseases of sheep and goats contribute to 5.6 percent of the total diseases of small ruminants. These infectious respiratory disorders are divided into two groups: the diseases of upper respiratory tract, namely, nasal myiasis and enzootic nasal tumors, and diseases of lower respiratory tract, namely, peste des petits ruminants (PPR), parainfluenza, Pasteurellosis, Ovine progressive pneumonia, mycoplasmosis, caprine arthritis encephalitis virus, caseous lymphadenitis, verminous pneumonia, and many others. Depending upon aetiology, many of them are acute and fatal in nature. Early, rapid, and specific diagnosis of such diseases holds great importance to reduce the losses. The advanced enzyme-linked immunosorbent assays (ELISAs) for the detection of antigen as well as antibodies directly from the samples and molecular diagnostic assays along with microsatellites comprehensively assist in diagnosis as well as treatment and epidemiological studies. The present review discusses the advancements made in the diagnosis of common infectious respiratory diseases of sheep and goats. It would update the knowledge and help in adapting and implementing appropriate, timely, and confirmatory diagnostic procedures. Moreover, it would assist in designing appropriate prevention protocols and devising suitable control strategies to overcome respiratory diseases and alleviate the economic losses.

1. Introduction

Small ruminants particularly sheep and goats contribute significantly to the economy of farmers in Mediterranean as well as African and Southeast Asian countries. These small ruminants are valuable assets because of their significant contribution to meat, milk, and wool production, and potential to replicate and grow rapidly. The great Indian leader and freedom fighter M. K. Gandhi "father of the nation" designated goats as "poor man's cow," emphasizing the importance of small ruminants in poor countries. In India, sheep and goats play a vital role in the economy of poor, deprived, backward classes, and landless labours. To make this small ruminant based economy viable and sustainable, development of techniques for early and accurate diagnosis holds prime importance. Respiratory diseases of small ruminants are multifactorial [1] and there are multiple etiological agents responsible for the respiratory disease complex. Out of them, bacterial diseases have drawn attention due to variable clinical manifestations, severity of diseases, and reemergence

of strains resistant to a number of chemotherapeutic agents [2]. However, sheep and goat suffer from numerous viral diseases, namely, foot-and-mouth disease, bluetongue disease, maedi-visna, orf, Tick-borne encephalomyelitis, peste des petits ruminants, sheep pox, and goat pox, as well as bacterial diseases, namely, blackleg, foot rot, caprine pleuropneumonia, contagious bovine pleuropneumonia, Pasteurellosis, mycoplasmosis, streptococcal infections, chlamydiosis, haemophilosis, Johne's disease, listeriosis, and fleece rot [3–10].

The respiratory diseases represent 5.6 per cent of all these diseases in small ruminants [11]. Small ruminants are especially sensitive to respiratory infections, namely, viruses, bacteria, and fungi, mostly as a result of deficient management practices that make these animals more susceptible to infectious agents. The tendency of these animals to huddle and group rearing practices further predispose small ruminants to infectious and contagious diseases [6, 9]. In both sheep and goat flocks, respiratory diseases may be encountered affecting individuals or groups, resulting in poor live weight gain and high rate of mortality [5]. This causes considerable financial losses to shepherds and goat keepers in the form of decreased meat, milk, and wool production along with reduced number of offspring. Adverse weather conditions leading to stress often contribute to onset and progression of such diseases. The condition becomes adverse when bacterial as well as viral infections are combined particularly under adverse weather conditions [1]. Moreover, under stress, immunocompromised, pregnant, lactating, and older animals easily fall prey to respiratory habitats, namely, *Streptococcus pneumoniae*, *Mannheimia haemolytica*, *Bordetella parapertussis*, *Mycoplasma* species, *Arcanobacterium pyogenes*, and *Pasteurella species* [2, 4, 7–9, 12, 13]. Such infections pose a major obstacle to the intensive rearing of sheep and goat and diseases like PPR, bluetongue, and ovine pulmonary adenomatosis (Jaagsiekte) adversely affect international trade [2, 9, 10, 13], ultimately hampering the economy.

2. Respiratory Diseases of Small Ruminants

Depending upon the involvement of etiological agent, the infectious respiratory diseases of small ruminants can be categorized as follows [9, 14]:

(1) bacterial: Pasteurellosis, Ovine progressive pneumonia, mycoplasmosis, enzootic pneumonia, and caseous lymphadenitis,

(2) viral: PPR, parainfluenza, caprine arthritis encephalitis virus, and bluetongue,

(3) fungal: fungal pneumonia,

(4) parasitic: nasal myiasis and verminous pneumonia,

(5) others: enzootic nasal tumors and ovine pulmonary adenomatosis (Jaagsiekte).

Manytimes due to environmental stress, immunosuppression, and deficient managemental practices, secondary invaders more severely affect the diseased individuals; moreover, mixed infections with multiple aetiology are also common phenomena [5, 8, 13, 15].

These conditions involve respiratory tract as primary target and lesions remain confined to either upper or lower respiratory tract [7, 16]. Thus, these diseases can be grouped as follows [5, 8, 14, 17].

(1) Diseases of upper respiratory tract, namely, nasal myiasis and enzootic nasal tumors, mainly remain confined to sinus, nostrils, and nasal cavity. Various tumors like nasal polyps (adenopapillomas), squamous cell carcinomas, adenocarcinomas, lymphosarcomas, and adenomas are common in upper respiratory tracts of sheep and goats. However, the incidence rate is very low and only sporadic cases are reported.

(2) Diseases of lower respiratory tract, namely, PPR, parainfluenza, Pasteurellosis, Ovine progressive pneumonia, mycoplasmosis, caprine arthritis encephalitis virus, caseous lymphadenitis, verminous pneumonia, and many others which involve lungs and lesions, are observed in alveoli and bronchioles.

Depending upon the severity of the diseases and physical status of the infected animals, high morbidity and mortality can be recorded in animals of all age groups. These diseases alone or in combination with other associated conditions may have acute or chronic onset and are a significant cause of losses to the sheep industry [3, 10]. Thus, the respiratory diseases can also be classified on the basis of onset and duration of disease as mentioned below [3, 9, 14, 18]:

(1) acute: bluetongue, PPR, Pasteurellosis, and parainfluenza,

(2) chronic: mycoplasmosis, verminous pneumonia, nasal myiasis, and enzootic nasal tumors,

(3) progressive: Ovine progressive pneumonia, caprine arthritis encephalitis virus, caseous lymphadenitis, and pulmonary adenomatosis.

3. Need of Advanced Diagnostic Approaches

The potential losses due to respiratory diseases can be minimized by sound diagnostic approach along with sound management programme [15]. Any kind of compromise with the diagnostic and management approach would severely affect the health status of the flock [19]. Early, rapid, and effective diagnosis of the respiratory diseases in small ruminants is a challenge due to limited laboratory resources in African and Southeast Asian countries where a large small ruminant population gets decimated due to respiratory disease outbreaks [15, 16]. Conventional methods of diagnosis may be available more frequently but they usually take longer to yield results, and also their specificity and sensitivity may not be up to the mark. In recent past, many advanced, rapid, sensitive, and specific serological and molecular tests have been developed. These diagnostic methods have supplanted the conventional diagnostic procedures owing to their speed,

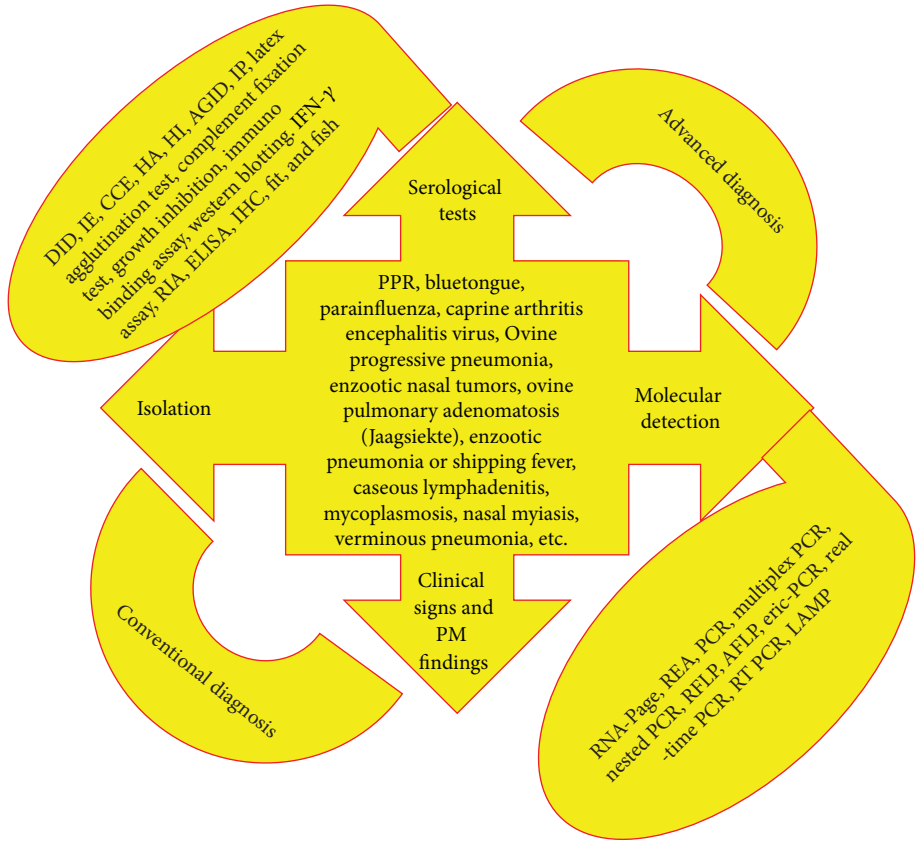

Figure 1: Diagnosis of infectious respiratory diseases of small ruminants.

sensitivity, specificity, and applicability even without isolation of etiological agent [20, 21].

In present scenario of globalization and regulations related to international trades, continuous monitoring of enlisted diseases is mandatory and for that sampling, isolation, and confirmation processes are very tedious [22, 23]. In such scenario, the rapid and specific detection of antibodies to the respiratory pathogens is now possible by the advancement in serological testing. Availability of better serological tests including ELISAs and monoclonal antibodies has enabled detection of antibodies to these infectious agents (namely, bacteria, viruses, and fungi) with more rapidity as well as specificity [24]. Moreover, due to advancement in the polymerase chain reaction (PCR) technology, there has been enormous improvement in the diagnosis of respiratory diseases of small ruminants [25]. Recent advances in biotechnology and molecular biology have led to the development of a variety of diagnostic assays, namely, PCR, RT-PCR, PCR-ELISA, RAPD, AFLP, RFLP, real-time PCR, quantitative PCR, multiplex PCR, LAMP, microsatellites, gene sequencing, and phylogenetic analysis, which not only help in identification but also assist in molecular characterization of various pathogens [20, 22–37]. Various conventional diagnostic tests, namely, isolation, postmortem finding, and gross clinical examinations along with modernized serological and molecular tests, are enlisted in Figure 1.

Advances in diagnostic tools and assays help strengthening the surveillance and monitoring systems of animal diseases. The latest advances in molecular techniques have assisted in the rapid and confirmatory diagnosis of the diseases and epidemiological studies to formulate appropriate and timely prevention, treatment, and control measures, and alleviation of economic losses to animal producers [1, 7, 13, 22, 23].

4. Advances in Diagnosis of Respiratory Diseases of Small Ruminants

For the prevention and control of fatal infectious respiratory diseases of small ruminants, various diagnostic strategies are adopted worldwide. The diagnostic tests as well as procedures adopted in different parts of world incorporate combination of conventional and advanced diagnostic tests. However, the initial suggestive diagnosis involves the observation of clinical signs and postmortem findings followed by serological and molecular methods for the confirmation of etiological agents. Common infectious respiratory diseases of small ruminants, clinical signs, postmortem findings, and diagnostic tests are compiled in Table 1.

4.1. Peste des Petits Ruminants (PPR). Peste des petits ruminants (PPR) is an acute and highly contagious viral disease of small ruminants and in particular of goats, popularly known

Table 1: Common infectious respiratory diseases of small ruminants.

Sl. no.	Name of condition	Etiological agents	Affected species	Clinical signs	PM findings	Diagnostic tests	References
1	Peste des petits ruminants (PPR)	*Morbillivirus* (family Paramyxoviridae)	Goats and sheep	Mucopurulent nasal and ocular discharges, necrotising and erosive stomatitis, enteritis, and pneumonia	Congestion of mucosa of respiratory tract, exudates in tract, hardening of lungs mainly in anterior lobes, congestion hemorrhages, and erosion in intestinal mucosa	HA, HI, ELISA, PCR-ELISA, RT-PCR, real-time PCR LAMP	[29, 30, 37–43]
2	Bluetongue	*Orbivirus* (family Reoviridae)	Goats and sheep	Swelling of the lips and tongue and typical blue coloration of tongue, though this sign is confined to a minority of the animals. Nasal symptoms may be prominent, with nasal discharge and stertorous respiration	In sheep-oedematous face and ears, with dry, crusty exudate on the nostrils; hyperemic coronary bands of hooves; petechial or ecchymotic hemorrhages may be present and extend down the horn. Petechiae, ulcers, and erosions in the oral cavity, particularly on the tongue and dental pad. The oral mucous membranes may be necrotic or cyanotic. The nasal mucosa and pharynx may be edematous or cyanotic, and the trachea hyperemic and congested. Froth is sometimes seen in the trachea	AGID, RNA-PAGE, FIT, RT-PCR, ELISA, and real-time PCR	[44–58]
3	Parainfluenza	Paramyxovirus	Sheep and goats	Mostly associated with enzootic pneumonia	Generally followed by bacterial infection mainly secondary *Mannheimia* infection	Direct immunofluorescence, ELISA, RT-PCR, real-time PCR, and multiplex PCR	[11, 18, 59–65]
4	Caprine arthritis encephalitis virus	Retrovirus	Neurologic disease in kids and arthritis in adults	Lymphocytic mastitis and hard firm udders along with chronic wasting	Signs of secondary bacterial infection: thickening of interalveolar septa and lymphoid hyperplasia, chronic interstitial pneumonia	TaqMan quantitative PCR (qPCR)	[18, 66–71]
5	Ovine progressive pneumonia (maedi-visna)	Oncogenic retrovirus of subfamily Lentiviridae	Sheep	Chronic emaciation, weakness, dyspnea, lymphoproliferative pneumonia, meningeal arteritis with encephalitis, nonsuppurative arthritis, and lymphocytic mastitis	Characteristic firm lungs with grayish to brown discoloration, thickening of interalveolar septa, and lymphoid hyperplasia	Immunohistochemical (IHC), cELISA, AGID, IP, and real-time quantitative PCR (qPCR)	[18, 72–76]

Table 1: Continued.

Sl. no.	Name of condition	Etiological agents	Affected species	Clinical signs	PM findings	Diagnostic tests	References
6	Enzootic nasal tumors, ovine pulmonary adenomatosis (Jaagsiekte)	Retrovirus	Sheep and goats	Signs of inspiratory dyspnea along with seromucoid nasal discharge	Presence of uni- or bilateral tumor growth; firm, hard, grey colored lungs; lungs sink in water, and bronchi are found filled with white frothy fluid	ELISA, RT-PCR, real-time PCR, IHC, and nested PCR	[77–83]
7	Enzootic pneumonia (Pasteurellosis, shipping fever, and hemorrhagic septicemia)	*M. haemolytica*and *Bibersteinia trehalosi (Pasteurella trehalosi)*	Sheep	Dyspnea, pyrexia, dullness, depression, mucopurulent nasal discharge, oculonasal blood, and tinged discharge	Serofibrinous fluid in lungs with fibrinous adhesions leading to consolidation of lungs	Counterimmunoelectrophoresis, ELISA, PCR, multiplex PCR, IHC, ISH, AFLP, AP-PCR, DNA fingerprinting, and Southern blot	[14, 84–96]
8		*M. haemolytica* and *P. multocida*	Goats				
9	Caseous lymphadenitis	*Corynebacterium pseudotuberculosis*	Sheep and goats	Chronic emaciation, dyspnea, exercise intolerance, dullness, forced full coughing, and weight loss	Enlargement of lymph nodes with greenish colored pus	Haemagglutination test, Counterimmunoelectrophoresis, IFN-γ ELISA, and PCR	[14, 97–103]
10	Mycoplasma	*M. ovipneumoniae*, *M. capricolum*, *M. mycoides* subsp. *mycoides*, and *M. agalactiae*	Sheep and goats, kids may develop encephalitis	Anorexia, pyrexia, painful breathing, coughing, and sneezing	Peribronchiolar lymphocytic infiltrations are observed with diffused nonsuppurative pleuritis	Immunoblotting, immunobinding assay, growth inhibition, PCR-RFLP, and multiplex real-time PCR	[7, 18, 104–119]
11	Nasal myiasis	*Oestrusovis*	Sheep	Stamping of feet, efforts to hide nose, difficult breathing, and heavy sound in respiration	Swollen nasal membranes, plugged nostrils, and upper respiratory tract occluded with serofibrinous discharge	Double immunodiffusion (DD), indirect haemagglutination (IH) tests, ELISA, and PCR PCR-RFLP	[120–125]
12	Verminous pneumonia	*Dictyocaulus filaria*, *Protostrongylus rufescens*, and *Muellerius capillaris*	Young animals in the age group of 2–18 months	Pyrexia, coughing, rapid and painful breathing, nasal discharge, and emaciation with retarded growth	Presence of parasites and caseous exudates in lungs	ELISA and PCR	[14, 126–130]

as goat plague [38, 39]. Transmission of the disease takes place by direct contact with the secretions or excretions from the infected animals to healthy ones, which are in close contact. Clinically, PPR is characterized by pyrexia, ocular and nasal discharges, erosive stomatitis, and diarrhea [38, 40]. The postmortem findings are limited mainly to the alimentary tract that consists of erosive stomatitis (extensive in nature) as well as hemorrhagic gastroenteritis. Often, streaks of congestion may be found along the mucosal folds that result in the characteristic appearance of "zebra-strip" [131, 132]. The morbidity and mortality rates of PPR can be as high as 100% and over 90%, respectively [39, 40].

The various serological tests applied in the PPR detection include agar gel immunodiffusion, virus neutralization, complement fixation, haemagglutination inhibition, and competitive ELISA assays. Conventional serological tests like complement fixation or haemagglutination inhibition cannot differentiate between PPR and Rinderpest (RP). However, haemagglutination inhibition (HI) can be used quantitatively for the measurement of PPRV antibodies in suspension. Titration of the PPRV antigen can be done by the use of both haemagglutination (HA) and HI tests [39–41]. Peste des petits ruminant's virus (PPRV) can be differentiated from Rinderpest (RP) by virus neutralization and competitive ELISA assays. Competitive ELISA can be a better choice for detection of antibody to PPR because of its high specificity of diagnosis [40]. A rapid as well as sensitive and virus-specific test for detection of PPRV antigen is immunocapture ELISA that can cause differentiation of RP and PPR. It has got higher sensitivity than routinely used agar gel immunodiffusion test [36, 42, 133].

There has been a substantial improvement in the techniques to detect the nucleic acids of PPRV. PCR assays are now considered as powerful as well as novel means of detection and quantification of the nucleic acids of PPR virus in various types of clinical samples. But unfortunately, no single assay can detect all the lineages of the virus. Companion tests can be developed by manipulation of the PPRV gene and insertion of either positive or negative markers [25, 29, 30]. The nucleoprotein based RT-PCR, which is based on nucleoprotein (N) genes, has been standardized recently. Instead of analysis of the amplified product by means of agarose gel electrophoresis, its detection is done on a plate by ELISA using labeled probe. The sensitivity of this RT-PCR ELISA is ten times higher than the classical RT-PCR. With the aid of quantitative real-time RT-PCR, there has been significant improvement in the diagnosis of PPR [29, 30]. This minimizes the risk of contamination. There is also description of applying nucleic acid amplification for the diagnosis of PPR. The sensitivity of this assay is similar to that of PCR but its simplicity in implementation, as the results can be read by naked eye, and rapidity make it suitable for practical application [32, 34].

Use of LAMP significantly reduced the processing time of sample and final outcome [21]. Similarly, LAMP assay based on conserved region of "N" gene of PPR virus has been documented for rapid and specific detection of PPR virus from clinical samples. The assay was found 100–1000 times superior to PCR and s-ELISA [43].

Synthetic peptide and multiple antigenic peptide based antigen has been employed in ELISA for detection of PPR virus antibodies. A PCR-ELISA based on N gene has been standardized in order to detect PPR virus thereby yielding a product (which is labeled with digoxigenin) that comprises a sequence from N gene of the PPRV. The assay has been found to be more sensitive than sandwich ELISA in order to detect the virus in both early and late phases of the disease. For differential diagnosis of PPRV from Rinderpest virus, also the assay has been found to be useful [27]. A one-step multiplex RT-PCR (single tube) has been standardized for amplification of specific fragments of the N as well as M genes of PPR virus. For detection of the virus directly from clinical field samples, the RT-PCR is conducted by the use of purified viral RNA. The assay is easier than the two-step assay as it is time saving requiring only one buffer for both reverse transcription and PCR [29]. RT-PCR based on F gene has shown a low sensitivity as well as specificity along with moderate agreement as compared to sandwich ELISA [36]. By the use of one-step Brilliant SYBR Green Kit, a sensitive as well as rapid single step real-time RT-PCR has been standardized for detection and semiquantitation of PPRV by the use of primers specific to viral RNA and matrix protein gene. They have been compared with conventional RT-PCR as well as Taqman RT-PCR. It has been found that the assay is more rapid as well as sensitive than TaqMan and the conventional RT-PCR in order to detect nucleic acid of PPRV from the clinical samples of sheep as well as goat, which are suspected for PPR. As an alternative test to the various diagnostic assays that already exist, SYBR Green RT-PCR has been found to be a successful tool thereby helping in rapid clinical diagnosis with advantage of reducing contamination risk [30, 37].

4.2. Bluetongue. Bluetongue (BT) is one of the important infectious diseases of domestic and wild ruminants. It is caused by bluetongue virus (BTV) of genus *Orbivirus* and family Reoviridae. The disease, transmitted by *Culicoides* (biting midges), was first reported in India in 1964 [134]. India has significant populations of domestic and wild ruminants, which are known to be susceptible to BTV infection. Several exotic breeds of sheep were introduced into the country between 1960 and 1970 for genetic improvement of the national flock by crossbreeding with native breeds [135]. This increase in the susceptible population, along with favorable climatic conditions, appears to have led to the establishment of BTV in the country [135, 136]. The disease has an incubation period of 5–20 days with the development of symptoms within a month. There is low mortality rate but in susceptible breeds of sheep the mortality may be high [136]. Asymptomatic infection is usually observed in cattle as well as goats and wild ruminants despite the high level of virus in the blood. Exception is red deer in which the disease may be as acute as in sheep [137]. The development in diagnostic technologies has confirmed over the past that BTV is now widely spread in several parts of India [136, 138].

Traditionally, the diagnosis of BTV is primarily based on clinical signs and symptoms. However, differential diagnosis

with some of the diseases such as contagious ecthyma, foot and mouth disease (FMD), vesicular stomatitis, malignant catarrhal fever (MCF), bovine virus diarrhea (BVD), infectious bovine rhinotracheitis (IBR), parainfluenza-3 infection, and sheep pox should be done [135, 136, 139]. The confirmatory diagnosis may be done either through virus isolation or through serological test. The virus isolation is performed in embryonated chicken eggs, in cell culture (BHK-21 or Vero cell line), or occasionally in sheep [139]. The virus is serotyped either by virus neutralization tests such as plaque reduction, plaque inhibition, Microtiter neutralization, and Fluorescence inhibition test (FIT) or through reverse-transcription polymerase chain reaction (RT-PCR) (a prescribed test for international trade) [50, 51]. A highly sensitive silver staining method of RNA-polyacrylamide gel electrophoresis (RNA-PAGE) of bluetongue virus was developed recently [49]. Various serological tests such as complement fixation test (now largely replaced by the AGID test), agar gel immunodiffusion, and competitive enzyme-linked immunosorbent assay (both are prescribed test for international trade) are used for serological characterization of BTV. Recently, novel Indian isolates of BTV 21 were detected employing real-time PCR assay [44]. The complete genome sequence of BTV serotype 16 of goat origin from India has also been carried out [140]. Similarly, the complete genome sequences of BTV22 and reassortment strain of BTV 2, 3, 16, and 23 from India have been carried out recently [45–48, 52]. Analyses of the nucleotide sequence as well as phylogenetic comparisons of genome segment 2 that encodes outer-capsid protein VP2 help in creation of segment-2 database [53]. Such database is used for developing rapid as well as reliable typing assay based on RT-PCR [50, 51, 54]. Testing of multiple primer pairs has also been done that provides an identification of serotype initially by amplifying a cDNA product of the expected size. Confirmation of serotype has been done by sequencing of the cDNA amplicons and subsequently phylogenetic analysis is done for comparing with reference strains that are previously characterized [52, 54]. The RT-PCR assay provides a rapid as well as sensitive and reliable method to identify and differentiate all the serotypes of BTV [45, 50, 51, 55–58].

4.3. Parainfluenza. Parainfluenza is mainly characterized at necropsy by purulent bronchopneumonia (focal) along with moderate to severe pulmonary congestion. Histopathological analysis has revealed the presence of acute and severe as well as diffuse necrotizing and fibrinous or suppurative bronchopneumonia. There is also a presence of diffuse congestion as well as pulmonary edema [61]. As a diagnostic method, comparison of enzyme immunoassay has been done with complement fixation test (CFT). The cross-reactivity of the viruses can be detected by the application of such tests [59]. Parainfluenza is a viral infection of the lower respiratory tract causing an enormous burden of disease in small ruminants. Direct immunofluorescence technique along with cross-neutralization tests is required for antigenic analysis of the parainfluenza virus isolates. For detection of the virus associated with it, new diagnostic test like multiplex PCR has got enormous advantages mainly because of its specificity [17]. Real-time PCR (RT-PCR) is a useful molecular tool for detection of parainfluenza virus type 3 (Pi3) from ribonucleic acid (RNA) samples from cells of the lungs from the slaughtered animals. This is followed by sequencing as well as restriction enzyme patterns of the fragment amplified of the F gene which confers confirmation of the distinctness of the isolates. Availability of suitable PCR primers allows detection of the ovine virus specifically [62]. Phylogenetic analysis of the amino acid as well as the nucleotide sequences is also equally important [60]. In some of the instances, it has been seen that the in-house RT-PCR methods cannot yield expected products for which the nucleotide sequence analysis has been initiated [63]. Multiplex RT-PCR can help distinguish parainfluenza viruses from other respiratory virus like adenovirus [64]. Nucleic acid sequence based amplification (NASBA) has been developed for which primers as well as probes have been selected from the haemagglutinin-neuraminidase (HN) gene as well as from the phosphoprotein (P) of the parainfluenza virus [61, 65].

4.4. Caprine Arthritis Encephalitis Virus. Caprine arthritis encephalitis virus (CAEV) is a member of the lentivirus family (in small ruminants) leading to chronic disease of the joints and rarely encephalitis in goat kids under the age of six months. The virus is in close intimation with white blood cells. Thus, any kinds of body secretions containing blood cells are potential sources for virus spread to other animals in the herd [141, 142]. In goats,in order to detect caprine arthritis encephalitis virus (CAEV), serological tests or cell cultures are mainly used. Besides, PCR has also been developed for detection of CAEV sequences from peripheral blood mononuclear cells (PBMC), synovial fluid cells (SFC), and milk cells (MC) from the infected goats. This type of PCR assay especially provides a useful method to detect CAEV infection in goats [66–68]. A two-step TaqMan quantitative (q) PCR, which is specific as well as sensitive for the detection of infection due to CAEV by the use of a set of primers (specific), and a TaqMan probe that targets a region which is highly conserved within the gene that encodes the capsid protein of the virus have been developed [33]. In the total deoxyribonucleotide (DNA) extracts, the proviral DNA can be detected successfully by this assay. The TaqMan qPCR assay provides a fast as well as specific and sensitive means for detection of proviral DNA of the virus and thereby proves to be useful for detection in large scale for eradication programs as well as epidemiological studies.

PCR techniques have been standardizedin several laboratories for the detection of proviral DNA. Other molecular techniques such as cloning and sequencing are also used to provide knowledge on a country or region's specific strain of CAEV. Phylogenetic analyses of the proviral DNAs of CAEV throughout the world have given the suggestion that in certain areas CAEV causes natural infection not only in goats but also in sheep. In order to track the transmission of the disease in near future, phylogenetic analyses may be used [66, 69, 70]. Molecular techniques such as cloning and sequencing are also used to provide knowledge on the prevalence of

specific strain of CAEV in a country or a region which may have influence on serological assay as well as corresponding CAEV antigen [33, 71].

4.5. Ovine Progressive Pneumonia (Maedi-Visna). Most of the sheep suffering from Ovine progressive pneumonia (OPP) do not show the clinical signs until the age of 2 years due to the long incubation period of the virus. General loss in body condition known as the "thin ewe syndrome" is the first sign of the disease. There may be loss of weight in spite of the normal appetite of the affected sheep [143, 144]. Several serological tests like agar gel immunodiffusion (AGID), immunoprecipitation (IP), and competitive ELISA (cELISA) are used for the diagnosis of Ovine progressive pneumonia with the use of methionine-labelled antigen A [73]. Real-time quantitative PCR (qPCR) which is specific for the transmembrane region of the envelope gene (tm) has been compared with competitive inhibition enzyme-linked immunosorbent assay (cELISA) using sheep sera. The qPCR assay indicates excellent agreement between the two tests. Both disrupted whole virus and recombinant viral proteins have been utilized in indirect ELISAs which have shown high sensitivity as well as specificity of detection [73]. Such experiments have proved that the proviral loads of Ovine progressive pneumonia virus (OPPV) qPCR can be confirmed by cloning as well as sequencing and can be used as diagnostic tool for OPPV infection as well as measurement of viral load in sheep which are infected [74, 75]. Single enzyme-based automated immunohistochemical (IHC) analysis has been developed to detect capsid antigen (CA) of OPPV that uses two anti-CAEV monoclonal antibodies, namely, 5A1 as well as 10A1 along with two enzyme-based IHC systems. The CA of OPPV has been detected in the intracellular regions of the synovial membrane of the carpus, in the cells that resemble alveolar macrophages as well as interstitial macrophages in the lung tissue, and so also in alveolar cells of the mammary gland [76]. Comparison of a new real-time quantitative PCR (qPCR) which is specific for the envelope gene's transmembrane region has been done with a competitive ELISA (cELISA). Such comparative test has led to the conclusion that qPCR may be used as a supplemental tool for diagnosis and for measuring the load of the virus [71, 145].

4.6. Enzootic Nasal Tumors and Ovine Pulmonary Adenomatosis (Jaagsiekte). From the diagnostic point of view of enzootic nasal tumors and ovine pulmonary adenomatosis, it is important to note that the genome of the ovine pulmonary adenomatosis virus is 7,434 nucleotides long thereby exhibiting a genetic organization of type B as well as D oncoviruses. The enzootic nasal tumor virus is closely related to the Jaagsiekte retrovirus of sheep as well as to sheep endogenous retroviruses [146, 147]. Diagnosis of enzootic nasal tumors is based on mainly clinical findings. Endoscopy reveals occlusion in the caudal part of one or both the nasal cavities. Radiography may also reveal the extent of the lesion. Provisional diagnosis can be made by the biopsy of the mass during the period of endoscopic examination [80]. RT-PCR for the diagnosis of Jaagsiekte is very important in order to formulate prevention as well as control strategies. The envelope (env) gene is mainly targeted for this purpose [81]. For development of an assay based on serology, identification of three proteins has been done as candidate diagnostic antigens, namely, Jaagsiekte sheep retrovirus (JSRV) p26 (which is a group specific antigen), the transmembrane, and the open reading frame (ORF)-X proteins. Isolation of the genes coding for all the three proteins has been done followed by cloning as well as expression. Purification of the JSRV p26 has been done as a potential diagnostic antigen by both Western blot and ELISA. Investigation of three molecular assays has been done for their sensitivity as well as specificity: the long terminal repeat (LTR) group specific antigen (gag) PCR, LTR heminested PCR, and the PCR covering the V1 or V2 region. The use of AmpliTaq gold DNA polymerase increases the specificity of heminested PCR. The complete genome sequence of the ovine enzootic nasal tumor virus has been done which has shown its exclusive association with contagious intranasal tumors of sheep [79, 82, 83].

4.7. Enzootic Pneumonia or Shipping Fever. Before discussing enzootic pneumonia in sheep, it has to be kept in mind that as far as the transmission of the disease from diseased to healthy animals is concerned, no direct evidence is available yet. As per suggestion, it has been noted that there may be precipitation of outbreaks due to abrupt environmental changes and it may also be associated with a sharp change in weather conditions [86, 87]. Such infection in animals caused by a bacterial species related to genus *Pasteurella* is known as Pasteurellosis. After the taxonomic revision in 1999, the species is classified as *Mannheimia species*. *Pasteurella multocida (P. septica)* is carried in mouth and respiratory tract of several animals, notably cats. The organisms are small Gram-negative bacillus with bipolar staining. *P. multocida*, a common commensal, causes numerous pathological conditions in domestic animals, avian species, and human beings. Pasteurellosis is associated with a close animal contact and may be transmitted by animal bite [88, 89]. Severe clinical conditions occur when the organism is associated with other infectious agents, such as mycoplasma, chlamydia, and viruses [7, 9]. Environmental conditions and various stress factors such as transportation, housing deficiency, and bad weather also play a role to further aggravate the clinical conditions. Among the various diseases considered to be caused by *P. multocida*, alone or in association with other pathogens, most important is shipping fever in cattle and sheep, which may also be caused by *Mannheimia haemolytica*, in the absence of *P. multocida*. Fresh samples are the prerequisites for isolation of *Pasteurella multocida* and subsequently demonstration of the bipolar staining characteristic. A wide range of media that can be used for isolation of the organism are blood and chocolate agar and casein/sucrose/yeast (CSY) agar with supplementation of 5% blood. Other media include dextrose starch agar as well as trypticase soy agar. For demonstration of the characteristic staining feature, methylene blue or Leishman's stain is usually used. For serotyping, the tests include rapid slide agglutination test as well as indirect haemagglutination

test (for capsular typing); for somatic typing an agglutination test; and agar gel immunodiffusion for both capsular and somatic typing. For the rapid identification of capsular type, counterimmunoelectrophoresis is an important diagnostic tool. Dot immunobinding assay, immunoblotting of outer membrane proteins of vaccine, and field isolates of *Pasteurella multocida* have been used for rapid diagnosis [90, 91]. Comparative analysis of the outer membrane protein profiles of haemorrhagic septicaemia associated *P. multocida* by immunoblotting studies indicated that the major OMP of *P. multocida* (B: 2) is highly antigenic and 37 kDa OMP has potential for protective and immunodiagnostic studies [92].

In clinical samples as well as bacterial cultures, detection of organisms can be done by PCR. The pair of primers for this particular assay can amplify a 353 base pair (bp) fragment of the 16srRNA gene, which ultimately results in the amplification of DNA. Thus, this kind of PCR assay usually represents a valuable tool for diagnosing the disease early ultimately facilitating better control of the disease. Similar strategies can be adopted for the identification and confirmation of enzootic pneumonia in sheep with advanced molecular methods [20, 35].

For epidemiological investigations, characterization of isolates can be done by DNA fingerprinting but availability of such diagnostic test is restricted to research laboratories [85, 93]. Southern hybridization can lead to confirmation of the presence of the bacterial sequence, which is often suggestive of the virulence of the organism [94]. Upon presumptive or definitive diagnosis, further differentiation of isolates can be achieved by genotypic fingerprinting methods. Restriction endonuclease analysis for characterization of serotypes of hemorrhagic septicaemia can be done with the enzyme HhaI. Discrimination of the isolates can be done by application of ribotyping as well as large DNA separation by means of pulsed-field gel electrophoresis. The rapidity as well as reproducibility of AFLP is high with higher index of discrimination. PCR fingerprinting is feasible in any laboratory, which has got the PCR capability. RAPD analysis as well as arbitrarily primed PCR (AP-PCR) is found to be useful for epidemiological investigation. For discriminating sheep as well as goat isolates, repetitive sequence PCR is also found to be useful. Repetitive extragenic palindromic REP-PCR as well as single prime PCR has been found to be useful for differentiating various serogroups of the bacteria [95, 96].

4.8. Caseous Lymphadenitis. The disease is caused by *Corynebacterium pseudotuberculosis*. There are two basic forms of caseous lymphadenitis, that is, internal form and external form. Most of the affected animals manifest both forms of the disease depending on the multiple factors that are age, physiological conditions, environmental factors, and managemental practices [148]. There is obvious nodule formation under the skin as well as enlargement of peripheral lymph nodes in the external form. The affected lymph nodes along with the subcutaneous tissues are enlarged with thick as well as cheesy pus which may rupture outward spontaneously or during the process of shearing or dipping. The internal form of caseous lymphadenitis (CLA) is manifested by vague signs such as weight loss, poor productivity, and decrease in fertility [3, 148, 149]. For the detection of the causative agent, *Corynebacterium pseudotuberculosis*, in sheep and goats, a double antibody sandwich ELISA has been developed, which has been further modified for improving the sensitivity. The main objective of developing this test is to detect the presence of antibodies against the bacterial exotoxin. It has been found that six proteins with varying molecular mass ranging from 29 to 68 kilo Dalton (kDa) react with sera from both goats and sheep acquiring infection experimentally or naturally. For classification of the sera with inconclusive results, immunoblot analysis has been found to be valuable [100, 101]. Quantification of interferon gamma (IFN-γ) is essential for accurate diagnosis of the disease for which an ovine IFN-γ ELISA has been developed. The sensitivity of the assay is slightly more for sheep than in goats while the specificity of the assay is higher for goats than for sheep. It can thus be concluded that IFN-γ is a potential marker in order to determine the status of CLA infection in small ruminants [102]. For the diagnosis of CLA, another novel strategy is the employment of PCR for identification of the bacteria isolated from abscesses [103]. The PCR has been found to be both sensitive and specific in addition to its rapidity of detecting *C. pseudotuberculosis* from sheep that are naturally infected [99].

4.9. Mycoplasmosis. As far as the antigenic variation is concerned, mycoplasmas have complex mechanisms enabling them to evade the immune system. They thereby cause several clinical symptoms which are having significant economic effect on production of small ruminants [107]. There are many species in genus *Mycoplasma* associated with pneumonic and respiratory conditions in small ruminants, namely, *Mycoplasma agalactiae*, *Mycoplasma mycoides subspecies mycoides*, *Mycoplasma bovis*, *Mycoplasma capri*, *Mycoplasma capripneumoniae*, *Mycoplasma capricolum*, *Mycoplasma putrefaciens*, and many others [7, 9, 104, 106–108]. Mycoplasmainfection associated syndromes range from septicemia (acute) along with death to chronicity of infection that results in reduced production [150]. Pneumonia accompanied by mastitis, keratoconjunctivitis, abortions, and arthritis is commonly observed in mycoplasma syndrome [7, 9, 151]. The conventional methods for diagnosis of mycoplasmosis include isolation of caprine and ovine mycoplasma in modified Hank's Balanced Salt Solution Liquid Media (MBHS-L), followed by biochemical characterization and staining [7, 9, 105]. Initially, serological tests like growth inhibition, agar gel immunodiffusion, counter current electrophoresis, complement fixation, PAGE, and others were performed [110]. However, cross-reactivity of closely related species could not be differentiated by these serological tests [7–9, 104]. Immunobinding assay with polyclonal sera was able to differentiate closely related species [111]. It was followed by preparation of different antigens and purification with PAGE and SDS-PAGE in an attempt to identify potent specific immunogenic proteins of diagnostic values [112, 113]. Moreover, detection of protective and cross-reactive proteins with SDS-PAGE and immunoblotting showed some

glimpse of diagnostic value [9, 114, 115, 151]. These proteins provided base for selective and specific tests. Development of monoclonal antibodies based on such purified and specific immunogenic proteins led to development of very sensitive and specific sandwich ELISA based on monoclonal antibodies [116]. Molecular detection of *Mycoplasma* species based on different set of primers was used to identify different species [26]. For the development of monoclonal antibody based serological as well as ELISA-PCR, identification of species specific non-cross-reactive immunogenic proteins is mandatory, and for that proteins separated in SDS-PAGE were subjected to western blotting with homo- and heterologous sera against *Mycoplasma agalactiae* and *Mycoplasma bovis* [9, 114, 115, 151]. These species specific immunogenic proteins can form the basis for development of many advanced diagnostic procedures for the detection of mycoplasma and its species confirmation.

Nowadays, for the molecular diagnosis of several clusters as well as groups, species specific primers along with restriction enzymes are used for confirmation of the agent by PCR as well as PCR-RFLP [107]. Still the combination of conventional and recently developed molecular methods is recommended for the identification and confirmation of contagious caprine pleuropneumonia (CCPP) in field outbreak [117]. For this purpose, growth inhibition test has been employed for identification of the agent followed by PCR. These two tests in particular detect two species of *Mycoplasma*, namely, *Mycoplasma capricolum* and *Mycoplasma putrefaciens* from nasal swab and lung cultures [118]. A multiplex real-time PCR has been developed for differentiation of the various *Mycoplasma* species of sheep and goat including *Mycoplasma agalactiae*. This assay particularly targets the two specific housekeeping genes, namely, polC and fusA considering which specific diagnostic primers and probes are to be developed [105, 106]. It is however important to note that the assay requires further assessment of clinical specimens but for diagnosis on large scale basis the assay is very promising [119]. Primers specific to *Mycoplasma conjunctivae* (that causes pink eye in sheep and goat) have been used for amplification of a 750-base-pair fragment of the genome through PCR, which has been subsequently confirmed by agarose gel electrophoresis [107, 109].

4.10. Nasal Myiasis. Both double immunodiffusion (DD) and indirect haemagglutination (IH) tests are used for detection of the somatic crude antigen first (L1) as well as second (L2) and third (L3) in star of the larva of the parasite *Oestrus ovis* [122]. For postmortem examination, sagittal sectioning of the head of the sheep suspected of suffering from nasal myiasis is carried out for detecting the presence of maggots or larvae [152]. It has been observed that there is no development of cross-immune reaction in sheep, which are naturally parasitized with all the three larval stages (as detected by DD test) and with L2 larvae (as detected by IH test) [124]. It is important to note that rhinoscopy examination can confirm the diagnosis and is equally important in treating the patient by removing the maggots with forceps [123]. For detection of seropositivity, ELISA is employed using a crude L2 larva as antigen [121]. Development of a direct ELISA by the use of a crude somatic antigen was developed from the first stage larva (L1). Validation of such system has been done with sera from both endemic and nonendemic areas [125]. The sensitivity as well as the specificity of the assay has been found to be high by the use of a cut-off point. PCR as well as automated sequencing technologies have been developed for molecular diagnosis of the disease [128]. PCR-RFLP has been used widely for identifying taxa of the parasite which are closely related and have forensic relevance [120]. It is also important to note that a better understanding of several target genes like mitochondrial DNA (mt DNA) as well as ribosomal DNA (rDNA) is pertinent for understanding the evolution of the parasite and so also for characterization of the proteins of the parasites [120, 123].

4.11. Verminous Pneumonia. In goats *Muellerius capillaris* is the most common lung worm. There is diffused pneumonia in affected goats without the presence of any nodular lesion. The parasite predisposes animals to secondary infections thereby compromising with the health in general [129]. A rapid as well as inexpensive method for assessment of herd exposure to lung worm in cattle is the bulk milk ELISA. It is a useful tool for the veterinary practitioners as a herd health monitoring programme component or in the perspective of investigation of herd health [126]. Over the past 15 years, studies have been conducted to prove that sequences of the internal transcribed spacers of ribosomal DNA provide useful genetic markers. This makes the basis for the molecular diagnosis of parasitic pneumonia in sheep and goat using PCR [130]. DNA probes as well as assays based on PCR are used for identification and detection of *Dictyocaulus* as well as *Protostrongylus*. The sensitivity of most of the PCR-based assays is more than DNA probe assays. Multiple steps are required for the development of assays based on PCR, which follows the selection of oligonucleotide primers at the initial stage along with reporter probe. It has been found that usually PCR detects the parasitic DNA but certainly advances have been made in preparing samples. For this purpose, it is required to extract the DNA while removing the PCR inhibitors. This helps in achieving greater sensitivity [128].

5. Other Unusual Complications of Respiratory Tracts

The respiratory diseases of small ruminants are generally fatal to lambs and kids. The lamb and kid pneumonia are mostly regarded as a complex of disease. It involves interaction of host related factors (immunological and physiological) and etiological agents, namely, virus, bacteria, mycoplasma, and environmental factors [4, 7, 108]. Many times, immunosuppression, malnutrition, and adverse climatic conditions lead to infection due to unusual infectious agents. There are reports on *Streptococcus pneumoniae*, commensal bacteria of the nasopharynx of animals associated with a majority of cases of morbidity and mortality in young lambs due to pneumonia [7, 13, 153]. Similarly, many other unusual

pathogens *Haemophilus ovis* [154], *Streptococcus* spp., *Pasteurella* spp. [5, 6], *M. bovis* in sheep [7, 12] and goat [155], *Mycoplasma arginini* [12], and *Haemophilus somnus* [156] may cause pneumonia. Many time mixed infections are observed. Thus, isolation and identification of such samples are always tedious to perform [7, 9]. The use of monoclonal antibodies based serological tests has simplified the process of early and specific diagnosis of many of these pathogens [24]. Simultaneously, development of molecular techniques like PCR particularly multiplex PCR is very useful for the identification and differentiation of etiological agents from such complex conditions [21].

6. Conclusion and Future Perspectives

For effective control of respiratory diseases of sheep and goat, accurate diagnosis along with genetic characterization of the causative agents is essential. It is especially important in present context of increased antibiotic and anthelmintic resistance. The known limitations of the traditional diagnostic techniques have created urgency to give a boost to the development of molecular diagnostic techniques along with establishment of traditional serodiagnostic facilities for overall progress in the field of respiratory disease diagnosis. Advancement in development and standardization of various kinds of PCR techniques along with genetic characterization of the causative agent has provided a solid foundation to develop practical as well as highly sensitive and specific diagnostic tools to help conduct epidemiological investigation and devise control programmes. Multiplexing of PCR assays has decreased confusion of diagnosticians in case of mixed infection as such assays can certainly differentiate between the various species within the same genera of pathogenic organism. It is obvious that advancement in the field of bionomics and biotechnology has led to the rapid and accurate diagnosis of many of these economically important diseases. All such efforts ultimately will lead to improvement of economic status of stakeholders of small ruminant husbandry and their sustainability. Development in field of nanotechnology has led to evolution of nanomedicine with the aid of which it will be certainly possible in near future to make further progress in the diagnosis and management of the respiratory diseases of small ruminants including wild life.

Conflict of Interests

The authors declare that there is no conflict of interests in the publication of this paper.

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Research Article

Antibiotic Treatment Response of Chronic Lung Diseases of Adult Sheep in the United Kingdom Based upon Ultrasonographic Findings

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Examination of the lungs of adult sheep with chronic respiratory diseases was readily achieved using both 5 MHz linear and sector scanners. Superficial lung abscesses in eight sheep appeared as anechoic areas containing multiple hyperechoic dots bordered distally by a broad hyperechoic capsule. Unilateral fibrinous pleurisy (2 sheep) appeared as an anechoic area containing a hyperechoic latticework. Ovine pulmonary adenocarcinoma (OPA) lesions appeared as sharply demarcated hypoechoic areas in the lung parenchyma initially in the cranioventral lung lobes (21 sheep) with lesions also present in the caudodorsal diaphragmatic lobe (11 sheep); abscesses and areas of calcification within the OPA tumour mass were also identified. Daily treatment with procaine penicillin for 30 consecutive days was successful in both sheep with unilateral fibrinous pleurisy and six sheep identified with superficial lung abscesses measuring 2–8 cm in diameter; only one of two sheep with more extensive lesions recovered. Auscultation of the chest failed to detect adventitious sounds in any of the ten sheep with lung abscesses; normal breath sounds were reduced over the area of fibrinous pleurisy; no pleuritic rubs were heard. Wheezes and crackles auscultated in some OPA cases and did not correlate well with lesions detected ultrasonographically.

1. Introduction

An aetiological approach to the diagnosis, treatment, and control of respiratory diseases affecting sheep is often adopted in review articles [1] and book chapters [2, 3] but such precise classification is unrealistic in most general veterinary practice situations because of cost and access to specialised laboratory facilities. Clinical signs vary with the stage of the respiratory disease process and are not pathognomonic for particular aetiological agents [4]. Terms such as "chronic pasteurellosis" are commonly used to describe respiratory disease associated with weight loss with the recommendation of oxytetracycline therapy but there is little clinical evidence for such a diagnosis. Furthermore, detailed gross necropsies undertaken on farm prove difficult to interpret especially when complicated by autolytic change; histopathological examination is not often undertaken for cost reasons.

Individuals with chronic respiratory diseases are in poorer body conditions and have a higher respiratory rate than other sheep in the group [5]. The accuracy by which auscultation of the chest, as part of the standard veterinary examination, can detect, localise, and differentiate lung pathology has been questioned following comparison of adventitious sounds auscultated over normal lung areas and lesions of OPA [6]. Moderate to severe coarse crackles detected in advanced cases of OPA were audible over a larger area than lesion distribution identified during ultrasound examination and confirmed later at necropsy [5].

Publication of auscultated sounds recorded over specific respiratory tract pathologies, defined during simultaneous ultrasonographic investigation, has allowed clinicians to assess the value of auscultation of the chest performed as part of the standard veterinary clinical examination of sheep [5, 7] and cattle [8]. Auscultation did not detect any

abnormal sounds in sheep with lung abscesses; unilateral pyothorax and marked fibrinous pleurisy caused attenuation of sounds relative to the contralateral normal lung. No sounds resembling the description of pleural frictions rubs were heard in cases of marked fibrinous pleurisy [5].

While ultrasonographic examination of the chest in cattle and sheep has been routinely undertaken in some veterinary schools for many years [9], this adjunct to clinical examination also has great potential in farm animal practice because the examination takes only 5 minutes and does not involve any laboratory fees. Many chronic respiratory infections of ruminants are not accurately diagnosed, and such cattle and sheep do not receive the correct antibiotic treatment [10, 11].

This paper describes the antibiotic treatment response in sheep with chronic lung diseases where the diagnosis was based upon ultrasonographic examination of the lungs using 5 MHz linear and sector scanners; no account was taken of auscultation findings. Lung pathologies were confirmed in those animals that failed to respond to treatment and were euthanased for welfare reasons. Necropsy examinations allowed comparison between sonographic findings and pathological changes.

2. Materials and Methods

The thirty one sheep included in this study originated from the University of Edinburgh's first opinion ambulatory practice and cases were referred by local veterinary practices. This study covered a two-year period (October 2011–October 2013 inclusive). To mimic the time constraints faced by farm animal practitioners, ultrasonographic examination of both sides of the chest, including skin preparation, totalled no more than 5 minutes for each type of ultrasound scanner and often took less time. Ultrasonographic examination of the chest was undertaken using both 5.0 MHz sector and linear transducers connected to a real-time, B-mode ultrasound machine (Aloka and BCF Technology Miniscan).

A 5 cm wide strip of skin was shaved on both sides of the thorax extending in a vertical plane from the point of the elbow to the caudal edge of the scapula corresponding to the 6th or 7th intercostal spaces. The prepared skin overlying the chest wall can be freely moved 3 cm which allowed examination of the caudal aspect of the lung field. The skin was soaked with warm tap water then ultrasound gel liberally applied to the wet skin to ensure good contact. The transducer head was firmly held at 90° to the skin overlying the intercostal muscles of the 6th or 7th intercostal spaces and the thorax was examined in the longitudinal (vertical) plane. The relatively large linear probe head was held on the chest wall at a slight angle to represent the angle of the intercostal spaces. It was important to visualise the echogenic (white) line of the normal visceral pleura at the most dorsal margin of the lung field before scanning the ventral areas of the chest. The visceral pleura was followed down the chest wall to identify the junction between normal lung and pathology where present.

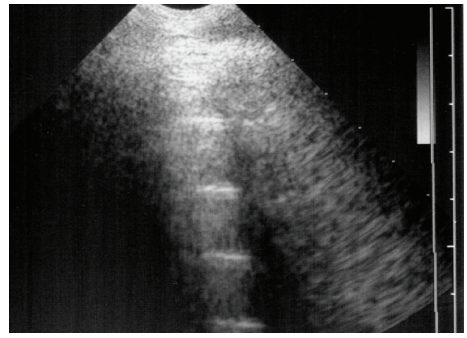

Figure 1: 5 MHz sector scanner. The probe head is at the top of the image; dorsal is to the left. Centimetre gradations are indicated on the margin. The surface of normal aerated lung (visceral or pulmonary pleura) of normal sheep is characterized by the continuous white linear echo. Equally-spaced reverberation artefacts are often visible below the visceral pleura.

The diagnosis of respiratory disease was confirmed at gross necropsy in 22 sheep. Bacteriology examinations were not undertaken because of prior antibiotic therapy. Ultrasonographic examination of 31 adult sheep with respiratory diseases associated with weight loss included OPA (21), fibrinous pleurisy (2 cases), and superficial lung abscesses (8 cases).

3. Results

3.1. Interpretation of Ultrasonographic Findings. The sonograms are presented with the probe head at the top of the image; dorsal is to the left and ventral to the right of the image. Centimetre dot markers are displayed on the margin of the images and should be consulted to ascertain the depth of field presented. The chest wall of adult sheep was approximately 1–1.5 cm thick. An air interface, created by aerated lung parenchyma reflects sound waves and appears as a bright white (hyperechoic) linear echo. The sonogram below the white linear echo may contain equidistant reverberation artefacts which are of no clinical significance. The area visualized below the linear echo, including the reverberation artefacts, does not represent lung parenchyma; thus the initial ultrasound machine setting with a 5 MHz sector transducer was 8 cm which examined approximately 1–1.5 cm of chest wall then pleurae and superficial lung parenchyma. The 5 MHz linear transducer used had a field depth of 7–9 cm.

The surface of normal aerated lung (visceral or pulmonary pleura) was characterized by the uppermost white linear echo with equally-spaced reverberation artefacts below this line (Figure 1). Careful placement of the large linear probe head was necessary to avoid the ribs (Figure 2). In normal adult sheep (around 50–80 kg) the visceral pleura was observed moving approximately 3 mm in a vertical plane during respiration. Comet-tail artefacts represent a series of closely-spaced discrete echoes indicating the focal accumulation of a small amount of highly reflective material, often gas bubbles. The chest wall was approximately 1–1.5 cm wide.

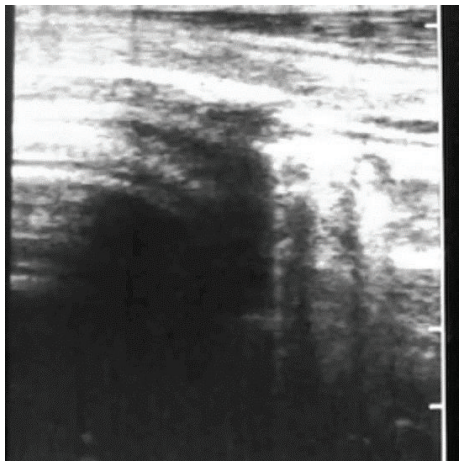

Figure 2: 5 MHz linear scanner. The dorsal (left) half of the probe head is positioned over a rib causing shadowing; normal lung surface is apparent ventrally (right).

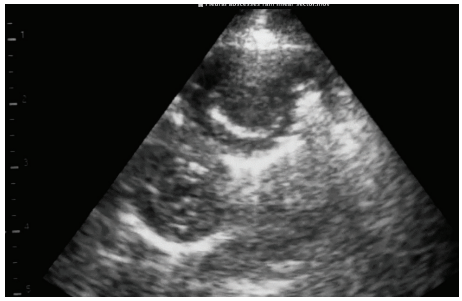

Figure 3: 5 MHz sector scanner. 2 cm diameter pleural abscess appear as anechoic areas containing numerous hyperechoic dots bordered distally by broad hyperechoic concave lines representing the capsules.

3.2. Superficial Lung Abscesses. The hyperechoic linear echo representing the normal visceral pleura was lost with superficial lung abscess which appeared as an uniform anechoic area containing many hyperechoic dots representing gas echoes bordered by a broad concave white abscess capsule (Figures 3 and 4). The abscess extended for 7 cm from the chest wall in Figure 4.

3.3. Fibrinous Pleurisy. The visceral pleura appeared broader and more hyperechoic than normal in one sheep due to acoustic enhancement by the pleural exudate (Figure 5) with fibrin extending to 3 cm present on the parietal and visceral pleurae. The visceral pleura could not be imaged in one sheep because the 7 cm wide anechoic area containing a hyperechoic fibrinous matrix extended beyond the depth range of the linear scanner (Figure 6).

3.4. Ovine Pulmonary Adenomatosis (OPA). The first indication of change in the lung parenchyma caused by OPA was the abrupt loss of the bright linear echo formed by normal aerated lung tissue (visceral or pulmonary pleura) to be replaced by a large hypoechoic area in the ventral margins of the lung

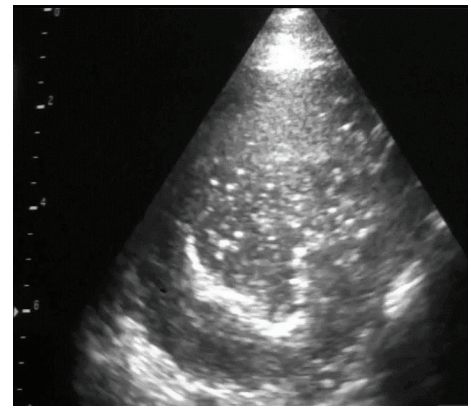

Figure 4: 5 MHz sector scanner. Sonogram of a large lung abscess which extends 8 cm into the lung parenchyma.

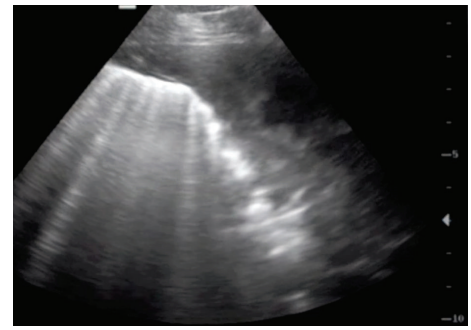

Figure 5: 5 MHz sector scanner. The visceral pleura appears broader and more hyperechoic than normal due to acoustic enhancement by the pleural exudates with fibrin extending to 3 cm present on the parietal and visceral pleurae.

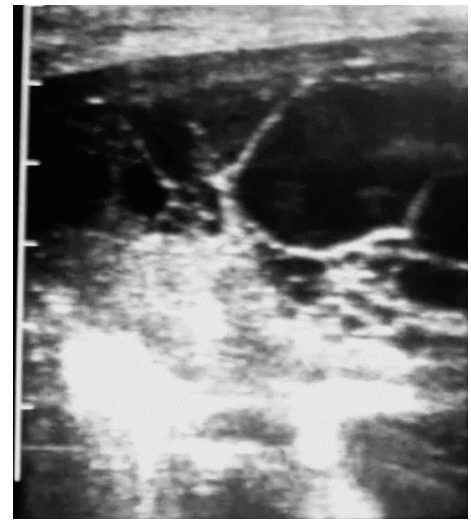

Figure 6: 5 MHz linear scanner. The anechoic area containing a hyperechoic fibrinous matrix extends beyond the 7 cm depth range of the linear scanner.

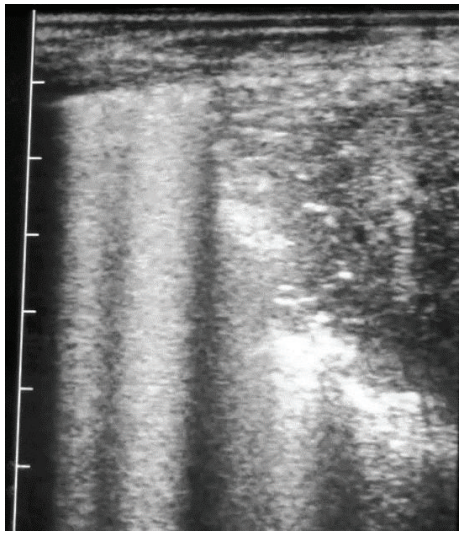

Figure 7: 5 MHz linear scanner. Abrupt loss of the bright linear echo formed by normal aerated lung tissue (visceral or pulmonary pleura) to be replaced by a large hypoechoic area in the ventral lung.

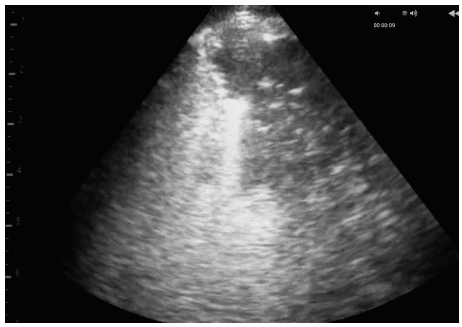

Figure 8: 5 MHz sector scanner. Abrupt loss of the bright linear echo formed by normal aerated lung tissue (visceral or pulmonary pleura) to be replaced by a large hypoechoic area in the ventral lung.

lobes at the 5th or 6th intercostal spaces (Figures 7 and 8). The dorsal margin of sonographic change representing that the extent of the OPA lesion was similar for either 5 MHz linear or sector scanners. The hypoechoic areas, corresponding to lung tissue invaded by tumour cells causing consolidation (Figures 7 and 8), allowed the distribution of the OPA lesions to be accurately defined during the ultrasonographic examination. Focal hyperechoic areas, identified within the more cellularly-dense hypoechoic areas, represented large airways. Abscesses were readily identified within the tumour mass of several OPA cases; shadowing was attributed to fibrosis/calcification of necrotic centres within the tumour (Figure 9).

Consecutive daily treatment with procaine penicillin for 30 days was successful in all six sheep identified with pleural/superficial lung abscesses measuring 2–8 cm in diameter; only one of two sheep with more extensive lesions recovered.

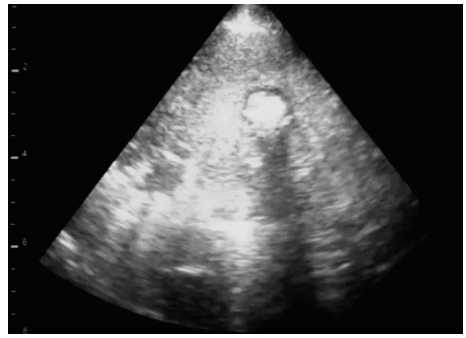

Figure 9: 5 MHz sector scanner. 2 cm diameter abscess dorsally (to left) within the OPA tumour mass; shadowing was attributed to fibrosis/calcification of a necrotic centre within the OPA lesion.

4. Discussion

Chronic bacterial infection of the respiratory tract in sheep usually presents with weight loss over several weeks/months and an increased respiratory rate [11]. A wide range of descriptors has been used to describe abnormal lung sounds in sheep including increased vesicular sounds for a ram with severe chronic suppurative pleuropneumonia [12] and wheezing, rubbing vesicular, and murmuring sounds in sheep with bacterial respiratory infections, followed by absence of residual bronchial catarrh in the same sheep during recovery [13]. Authors in more recent papers [14, 15] have limited their descriptions of abnormal auscultation findings of the respiratory tract to their distribution rather than character. The present study concurs with earlier published findings [5] that auscultation does not detect any abnormal sounds in sheep with lung abscesses; marked fibrinous pleurisy caused attenuation of sounds relative to the contralateral normal lung and no sounds were heard resembling the description of pleural frictions rubs.

Ultrasonographic examination of the chest accurately defined superficial lung pathology in agreement with previous work [16, 17]. Ultrasonography was most helpful in the definitive diagnosis of superficial lung abscesses where the anechoic areas containing multiple hyperechoic dots bordered distally by a broad hyperechoic capsule were readily detected but generated no adventitious lung sounds. Daily treatment with procaine penicillin for 30 days was successful in all six sheep identified with pleural/superficial lung abscesses measuring 2–8 cm in diameter; only one of two sheep with more extensive lesions recovered. Success was defined as a rapid return to normal appetite, marked improvement in body condition, and normal respiratory rate. Penicillin is the antibiotic of choice for chronic respiratory disease in cattle and sheep because of the frequent isolation of *Arcanobacterium pyogenes* [11, 18]. *Arcanobacterium pyogenes* was the most common bacterial isolate from chronic suppurative pneumonia cases in cattle and such chronic infections were treated with a 4–6-week course of procaine penicillin with reasonable success [8]. A 4–6-week duration of daily penicillin injections is necessary because of the chronicity of infection and time-dependent action of this antibiotic

[11]. Other antibiotic treatments could include ceftiofur, amoxicillin, and amoxicillin/clavulanic acid combination but these regimens would prove considerably more expensive.

Lesions of ovine pulmonary adenocarcinoma (OPA) were sharply demarcated sonographically from normal lung where the hypoechoic areas extended 6–8 cm into the lung parenchyma in the cranioventral lung lobes and had the sonographic appearance of liver (hepatoid change). Such lesions cannot be accurately delineated by auscultation findings alone [5, 6]. The ultrasonographic diagnosis of OPA was confirmed at necropsy in all 21 cases in the present study; there were no false positive diagnoses. Ultrasound examination may play an important role in identifying OPA lesions and help prevent spread in the UK both for economic and animal welfare reasons.

5. Conclusions

Accurate identification and distribution of pleural and superficial lung pathology necessitated ultrasonographic examination; auscultation failed to identify common lesions including OPA. With some experience, systematic ultrasound examination of the ovine chest takes no more than 5 minutes. Long-term penicillin therapy was successful in 7 of 8 cases of pleural/superficial lung abscesses.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

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Research Article

Electrophoretic Analysis of Indian Isolates of *Mycoplasma agalactiae* and *Mycoplasma bovis* by SDS-PAGE and Immunoblotting

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Mycoplasma agalactiae and *Mycoplasma bovis* both are responsible for respiratory conditions in sheep and goats. *M. agalactiae* is a major pathogen of sheep and goats and accounts for almost 90% of outbreaks of contagious agalactia syndrome in goats and almost 100% in sheep. On the basis of clinical signs and cultural, morphological, and biochemical characterization it is almost impossible to differentiate between both the species. Moreover, due to presence of genomic and proteomic similarity most of the time routine diagnostic tests fail to differentiate between them. Hence the present study was conducted to find out the protein profile of isolates of both the species by SDS-PAGE and to find out the cross-reacting as well as differentiating immunogenic proteins by Immunoblotting, which can be of immunoprophylactic as well as diagnostic values. The study revealed 6-7 major immunogenic cross-reactive proteins with the presence of two important non-cross-reacting species specific polypeptides particularly 25.50 and 24.54 kDa in *M. agalactiae* and *M. bovis*, respectively, that might be of diagnostic values.

1. Introduction

Mycoplasmas are the smallest, simplest, and self-replicating organisms with the minimum set of organelles required for the growth and replication. Till now more than one hundred species of mycoplasmas [1, 2], widespread in nature as pathogens of human, animals, birds, fishes, reptiles, arthropods, and plants, have been recognized [1–3]. Out of these species *Mycoplasma agalactiae* and *Mycoplasma bovis* both are important pathogens of small ruminants [3–5]. *M. agalactiae* accounts for almost 90% of outbreaks of contagious agalactia syndrome in goats [6] and almost 100% in sheep [4, 7]. Contagious agalactia causes high economic losses due to loss in milk yield and kids/lambs because of abortions, neonatal deaths, and loss of animals [4, 8, 9]. In contrast to *M. agalactiae*, *M. bovis* is supposed to be a major pathogen in calf pneumonia complex and the isolation rates of it have been reported in the range of 23 to 35% [2, 10, 11]; however recently it has been observed in the respiratory infection of sheep with significant mortality [3]. *M. agalactiae* and *M. bovis* are two closely related species [11, 12] and were earlier regarded As subspecies of the same species [11]. Moreover, it is difficult to differentiate *M. agalactiae* and *M. bovis* on the basis of morphological, biochemical, and traditionally used serological tests due to many common antigens and receptors on cell surface for antibodies [5, 11–13]. Hence, the present study was designed to identify the non-cross-reactive as well as cross-reacting protein antigens of both the species as a prospective antigen for their detection through serological tests.

2. Material and Methods

2.1. Mycoplasma Strains. Two strains of *M. agalactiae* RPNS 216 and RPNS 200 isolated from pneumonic goats and one strain of *M. bovis* NC 317 were used in the present study.

2.2. Culture Media. The modified beef horse serum liquid (MBHS-L) medium for the propagation of *M. agalactiae* strains and liquid B medium for the revival and propagation of *M. bovis* strain were prepared according to the standard method [10]. For the colony characteristics solid media were prepared by the addition of 1.2% Bacto Agar (Difco) in respective liquid medium [10].

2.3. Whole Cell Antigens (WCA). WCA were prepared as per earlier prescribed method [14] with slight modifications. Actively growing 2 to 5 mL of *M. agalactiae* and *M. bovis* culture was inoculated in 10 mL liquid medium and incubated at 37°C for 48 hours. The growth was confirmed by the change in pH (change of color red to yellowish orange). This growth was subsequently transferred to larger volume of media and incubated for 4 to 5 days to obtain sufficient growth. Simultaneously the growth was checked for purity on Robertson Cooked Meat (RCM), Sabouraud's Dextrose Agar (SDA), and Blood Agar media. The growth was centrifuged at 10,000 rpm for 25 minutes using a refrigerated centrifuge (Sorvell, RC-5C). The pellets were washed thrice with PBS (pH 7.2) and finally suspended in 10 mL of PBS (pH 7.2). The protein concentrations of WCA were estimated by the standard method [15].

2.4. Sonicated Supernatant Antigen (SSA). Sonicated antigens of all the isolates were prepared from the whole cell antigen by the method described earlier [16] with slight modification. For the preparation of SSA from WCA, the whole cell antigens were diluted in PBS (pH 7.2) and sonicated with MSE-Soniprep 150 at 10 microns by applying 10 jerks, each of 90 seconds with the interval of 30 seconds, on ice. Thus prepared sonicated antigens were further centrifuged at 13,000 rpm for 30 minutes at 4°C (Biofuge, Fresco). The supernatant was separated carefully and was used as SSA. The protein concentration of SSA was estimated by the previously described procedure [15].

2.5. Production of Hyperimmune Sera. To assess the immunogenicity of WCA and SSA polypeptides through Immunoblotting, the polyclonal hyperimmune serum was raised against *M. agalactiae* (RPNS 216) and *M. bovis* (NC 317) in white New Zealand rabbits (obtained from LAR, IVRI, Izatnagar), according to the standard protocol [17]. These antisera were tested by slide agglutination test [18] and titer was estimated by indirect haemagglutination (IHA) [19] with slight modification [20]. Sera were finally filtered through 0.20 μm filter (Sartorius) and stored at −20°C for further use.

2.6. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). The SDS-PAGE of both (WCA and SSA) of *M. agalactiae* (RPNS 216 and RPNS 200) and *M. bovis* (NC 317), under denaturating, reducing conditions, was performed by the method of Lammli [21] with recommended modifications [14]. Electrophoresis was carried out using 12.5% separating and 4% stacking gel at 50 volts for 1 h and at 100–110 volts afterwards till the end of run. The determination of molecular weights was based on the distance migrated by the polypeptides in the gels in comparison to the distance migrated by polypeptide markers of known molecular weights (Biored) [22].

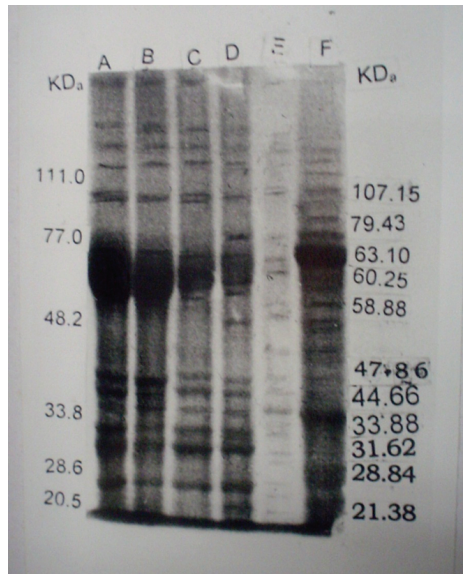

Figure 1: SDS-PAGE analysis of whole cell and sonicated supernatant antigens. Lane A: WCA of *M. agalactiae* RPNS 216. Lane B: WCA of *M. agalactiae* RPNS 210. Lane C: SSA of *M. agalactiae* RPNS 216. Lane D: SSA of *M. agalactiae* RPNS 210. Lane E: SSA of *M. bovis* NC 317. Lane F: WCA of *M. bovis* NC 317.

2.7. Immunoblotting. The WCA and SSA of all the isolates separated on 12.5% (w/v) SDS-PAGE slabs [21] were transferred electrophoretically on nitrocellulose membrane papers (NCP) (Sartorius). Then these membranes were cross-blotted with the hyperimmune serum raised against *M. agalactiae* (RPNS 216) and *M. bovis* (NC 317) separately by the previously described method [23] to find out cross-reactive polypeptides.

3. Results

The results of SDS-PAGE profiles of the two isolates of *M. agalactiae* and one of *M. bovis* have been presented in Figure 1. The molecular weights of the polypeptides in all the isolates ranged between 181.97 and 20.89 kDa (Figure 1). The number of polypeptides varied isolate to isolate and type of antigens used for SDS-PAGE (Figure 2). Both the isolates of *M. agalactiae* revealed 24 and 25 polypeptides in WCA and SSA, respectively, whereas WCA and SSA of *M. bovis* isolate revealed only 21 and 22 polypeptides, respectively. The patterns of major polypeptides were almost similar with minor differences. Profile of *M. agalactiae* isolates showed

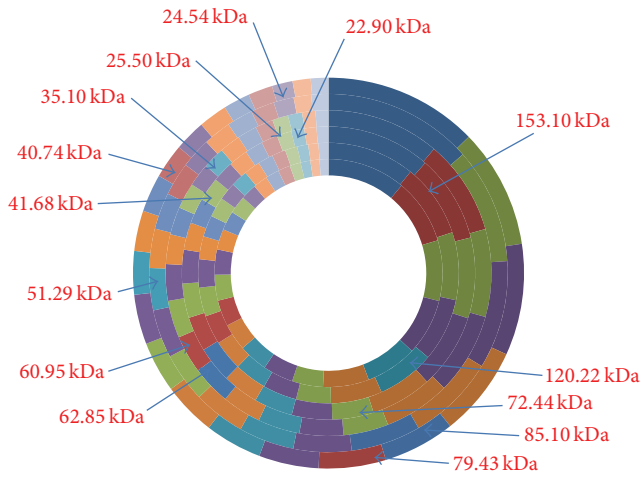

Figure 2: SDS-PAGE protein profiles have been depicted centripetally from *M. agalactiae* (RPNS 216) WCA; *M. agalactiae* (RPNS 216) SSA; *M. agalactiae* (RPNS 210) WCA; *M. agalactiae* (RPNS 210) SSA; *M. bovis* (NC 317) WCA; *M. bovis* (NC 317) SSA.

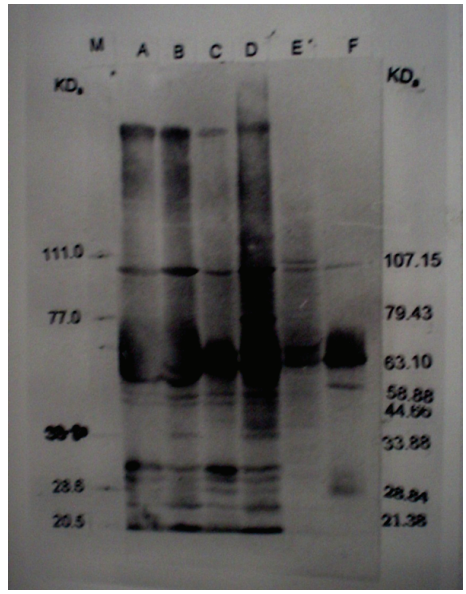

Figure 3: Immunoblot analysis of whole cell and sonicated supernatant antigens against *M. agalactiae* RPNS 216 hyperimmune sera. Lane A: WCA of *M. agalactiae* RPNS 216. Lane B: WCA of *M. agalactiae* RPNS 210. Lane C: SSA of *M. agalactiae* RPNS 216. Lane D: SSA of *M. agalactiae* RPNS 210. Lane E: SSA of *M. bovis* NC 317. Lane F: WCA of *M. bovis* NC 317. Lane M: Molecular weight marker.

the difference of two polypeptides; the polypeptide of 120.22 kDa was present in only SDS-PAGE profile of *M. agalactiae* RPNS 216 whereas polypeptide of 62.85 kDa was present in the profile of *M. agalactiae* RPNS 210 (Figures 1 and 2). The polypeptide of 35.10 kDa was present only in the SSA of both the isolates of *M. agalactiae* (Figures 1 and 2).

The SDS-PAGE profiles of *M. bovis* isolate also produced similar protein profile with almost similar migration patterns. However, the number of polypeptides appeared lesser in number (Figure 1) with 21 and 22 polypeptides in WCA and SSA, respectively (Figure 2). The polypeptide of 79.43 kDa was present only in SSA of *M. bovis*.

The polypeptides of molecular weights 153.10, 72.44, 60.95, 41.68, 25.50, and 22.90 kDa were present only in WCA and SSA of both the isolates of *M. agalactiae*. The polypeptides of 122.22, 62.85, and 35.10 kDa were absent from the protein profile of the *M. bovis* and were present exclusively in respective antigens of *M. agalactiae* (Figures 1 and 2). Moreover, the isolate of *M. bovis* was having exclusive polypeptide of 85.10, 51.29, 40.74, and 24.54 kDa along with 79.43 kDa in SSA only (Figures 1 and 2).

When Immunoblotting was performed with the hyperimmune serum against *M. agalactiae* RPNS 216 of *M. bovis* NC 317 the immunoblots revealed almost similar patterns of immunogenic polypeptides with the difference of some immunogenic polypeptides. As usual isolates revealed more immunogenic polypeptides with homologous hyperimmune serum, namely, 13 each in *M. agalactiae* isolates (Figures 3 and 5) and 13 and 14 in WCA and SSA of *M. bovis* (Figures 5 and 6). The numbers of cross-reactive antigens were comparatively lesser with 5 numbers of polypeptides in *M. agalactiae* isolates (Figures 5 and 6) and 7 in *M. bovis* isolate (Figures 3 and 4). The immunoblot profiles of both the isolates of *M. agalactiae* were identical with both homologous and heterologous hyperimmune serum (Figures 4 and 6). The immunogenic polypeptides of molecular

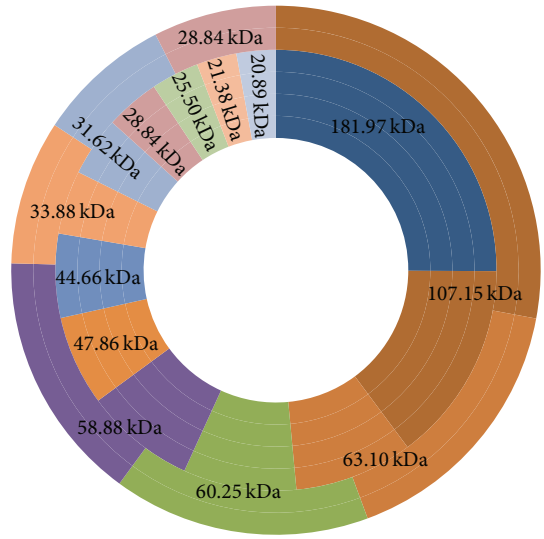

Figure 4: Immunoblot analysis depicted centripetally from *M. agalactiae* (RPNS 216) WCA; *M. agalactiae* (RPNS 216) SSA; *M. agalactiae* (RPNS 210) WCA; *M. agalactiae* (RPNS 210) SSA; *M. bovis* (NC 317) WCA; *M. bovis* (NC 317) SSA.

weights 47.86, 44.66, 33.88, 31.62, 21.38, and 20.89 were present in immunoblot of all the isolates but only with homologous hyperimmune serum (Figures 3, 4, 5, and 6). Only two polypeptides one each in *M. agalactiae* and *M. bovis* 25.50 and 37.15 kDa, respectively, were present exclusively in immunoblots of *M. agalactiae* and *M. bovis* with homologous hyperimmune serum (Figures 3, 4, 5, and 6).

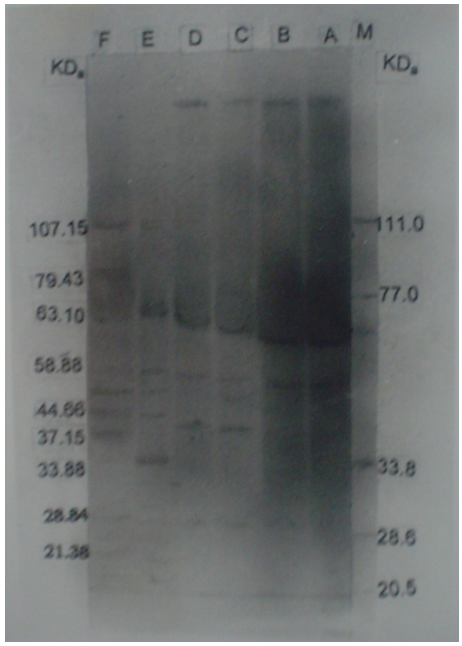

Figure 5: Immunoblot analysis of whole cell and sonicated supernatant antigens against *M. bovis* NC 317 hyperimmune sera. Lane A: WCA of *M. agalactiae* RPNS 216. Lane B: WCA of *M. agalactiae* RPNS 210. Lane C: SSA of *M. agalactiae* RPNS 216. Lane D: SSA of *M. agalactiae* RPNS 210. Lane E: SSA of *M. bovis* NC 317. Lane F: WCA of *M. bovis* NC 317. Lane M: molecular weight marker.

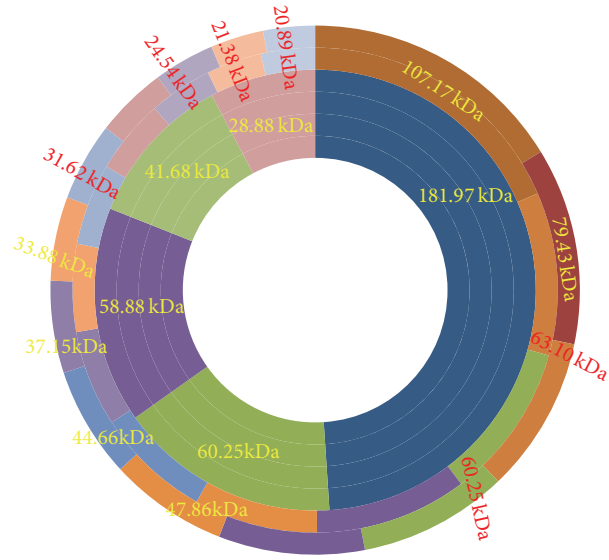

Figure 6: Immunoblot analysis depicted centripetally from *M. agalactiae* (RPNS 216) WCA; *M. agalactiae* (RPNS 216) SSA; *M. agalactiae* (RPNS 210) WCA; *M. agalactiae* (RPNS 210) SSA; *M. bovis* (NC 317) WCA; *M. bovis* (NC 317) SSA.

4. Discussion

The immunologic cross-reactivity among members of the mycoplasmatales is well documented and tests, namely, immunodiffusion, agglutination, two-dimensional immunoelectrophoresis, counter current immunoelectrophoresis, ELISA, immunobinding assay, polyacrylamide gel electrophoresis [1, 5, 11, 12, 24], and variety of other methods with the use of polyclonal serum, failed due to many common antigens and receptors on cell surface for antibodies [1, 5, 13]. The results of the present study are also in the concurrence of previous findings revealing many cross-reactive polypeptides [5, 11, 12, 24]. Moreover 52 and 60 common polypeptides were observed in almost similar range and pattern in *M. agalactiae* [25] and *M. bovis* [26, 27] with the presence of 5 to 7 major proteins in the range of 90 to 20 kDa. Similarly almost identical patterns with minor differences were recorded in *M. agalactiae* and *M. bovis* with one-third polypeptides of identical molecular masses [12]. However, in an earlier report, no common protein band but areas of homology did exist among different mycoplasmal species including *M. agalactiae* and *M. bovis* [13].

Based on previous information identification of species specific immunogenic proteins is always of diagnostic as well as prophylactic values. For that Immunoblotting, a sensitive and specific confirmatory test for the identification and isolation of immunogenic proteins was performed with homo- and heterologous hyperimmune serum [23]. The presence of 5–7 major immunogenic proteins (Figures 3, 4, 5, and 6) obtained during the Immunoblotting might be of diagnostic as well as protective values. These findings are in the concurrence of many earlier reports in various species of mycoplasmas *M. ovipneumoniae* [26], *M. mycoides* cluster [28], *M. hominis* [29], *M. hyopneumoniae* and *M. flocculare* [22], *M. agalactiae* [12, 14, 30], and *M. bovis* [12, 31, 32]. As protein patterns obtained by SDS-PAGE provide an indirect measure of the coding capacity of mycoplasmal genome and comparison sought to provide an approximate reflection of probable genomic capacity. However, each band may consist of many proteins of similar molecular weight as separation using SDS is dependent on molecular weight only [33]. Hence the presence of six to seven major immunogenic polypeptides in all the three isolates of both the species particularly the polypeptides of 60.25 and 28.84 kDa can be of immunoprophylactic use against both the species. Similarly the presence of species specific polypeptides particularly 25.50 and 24.54 kDa in *M. agalactiae* and *M. bovis*, respectively, might be of diagnostic values. However to authenticate the findings studies with more number of isolates from different geographical region are required.

5. Conclusions

It can be concluded from the present study that both the isolates of *M. agalactiae* have almost similar protein profile and presence of identical immunogenic polypeptides. Moreover, protein profile of *M. agalactiae* and *M. bovis* are almost similar with the presence of many cross-reactive major polypeptides. These can be differentiated by the presence of certain species specific immunogenic proteins or by using the monoclonal antibodies raised against those proteins in the diagnostics. However, a large number of isolates are required to be examined before these conclusions are put to practice.

Conflict of Interests

There is no conflict of interests among the authors.

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Review Article

Environmental Attributes to Respiratory Diseases of Small Ruminants

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Respiratory diseases are the major disease crisis in small ruminants. A number of pathogenic microorganisms have been implicated in the development of respiratory disease but the importance of environmental factors in the initiation and progress of disease can never be overemphasized. They irritate the respiratory tree producing stress in the microenvironment causing a decline in the immune status of the small ruminants and thereby assisting bacterial, viral, and parasitic infections to break down the tissue defense barriers. Environmental pollutants cause acute or chronic reactions as they deposit on the alveolar surface which are characterized by inflammation or fibrosis and the formation of transitory or persistent tissue manifestation. Some of the effects of exposures may be immediate, whereas others may not be evident for many decades. Although the disease development can be portrayed as three sets of two-way communications (pathogen-environment, host-environment, and host-pathogen), the interactions are highly variable. Moreover, the environmental scenario is never static; new compounds are introduced daily making a precise evaluation of the disease burden almost impossible. The present review presents a detailed overview of these interactions and the ultimate effect on the respiratory health of sheep and goat.

1. Introduction

Indian livestock sector has emerged as one of the key components of national as well as agricultural growth with an annual contribution of 3.93% (2,41,177 crore) of national GDP and 22.14% share in the agricultural GDP. Today, India ranks first with respect to buffalo, second in cattle and goats, and third in sheep population in comparison to the world livestock population [1]. It also provides self-employment opportunities to almost 6.7% of rural work force. Presently, livestock sector holds a substantial share in fulfillment of human food demand and this share is expected to further get doubled by 2030 [2]. To discharge this increasing demand of livestock products, it is essential that India increases the animal population, improves feed conversion efficiency, implements better reproductive policy, and overall improves the livestock health and productivity, that is, excess use of drugs as food additives, fattening agents, prophylactic antipathogenic drugs, boosters of reproductivity, and so forth. The attempt to increase livestock products (meat, eggs, and milk) production has also resulted in the production, accumulation, and dumping of large amounts of different kinds of wastes or pollutants in the environment all over the world. Aerosolization of microbial pathogens, endotoxins, drug residues, pesticides, offensive odour, and dust particles are all inevitable consequences of the generation and handling of waste material of the food production process, originating from animals. For optimizing livestock productivity, it is mandatory that small ruminant rearers realize that they form the front for identification and prophylaxis of entry of disease-causing agents (pathogens) into production systems [3–5] for a reduction in current on-farm vulnerabilities, upgrading food safety and food security, and enhancing their competence for production of a safer and wholesome product [6].

Broadly, the term "environmental pollution" refers to presence of any agent or a chemical in the environment of an individual which is potentially hazardous to either the environmental or individual's health. As such, environmental pollutants may take many forms: chemicals, organisms, and biological materials, as well as energy in its various forms (e.g., noise, radiation, and heat). The actual number of potential pollutants is therefore incalculable. Less than 1% of these pollutants have been subjected to a detailed appraisal in terms of their toxicity and health risks [7]. Furthermore, environmental conditions are never static; they undergo change over time and rare events may occur which may produce long-term health consequences in the exposed living populations. Such interactions between pathogens, their hosts, and novel environments may alleviate or compound the individual pathological responses, ultimately affecting its viability and contributing to insidious persistence or ultimate destruction of life. A suitable example may be the effect of abiotic factors which include insularity, climate, and volcanism on the prevalence and severity of disease in free-ranging sheep on Hawaii's Island [8].

Respiratory diseases are the major disease crisis in small ruminants [9, 10]. A number of epidemiological surveys have established the presence of the principal respiratory viruses and bacteria in majority of respiratory outbreaks. Repeated attempts have been made to tackle these outbreaks by prior vaccination but only limited success has been achieved. The present review discusses the contributions of environmental factors to initiation and progression of respiratory diseases in small ruminants.

2. Respiratory System of Small Ruminants

The respiratory tract of an adult goat comes into contact with approximately 7-8 liters of air per minute, that is, 11,000 liters of air in a day. Thus, the quality of inhaled air has major implications on the respiratory health of the animals. The respiratory system of sheep and goats is quite adaptable against a plethora of air contaminants [11] but disruption of defensive mechanisms to get rid of inhaled material may occur if an individual is exposed to highly concentrated particles in certain situations or if an exposure occurs during strenuous labour. Airborne contaminants may then serve as a primary cause of respiratory disease or can exacerbate a preexisting respiratory conditions or pulmonary disease. Depending on the inhaled substance, acute or chronic reactions occur as particles are deposited on the alveolar surface. Acute reactions are characterized by swelling (oedema) and inflammation [12], while chronic reactions are characterized by connective tissue scarring (fibrosis) and the formation of specific aggregates of immune cells (granulomas) [13]. Some of the effects of exposures may be immediate, whereas others such as lung disease related to asbestos deposits may not present for many decades [14].

3. Factors Affecting Development of Diseases

The production of disease in an animal is determined by three basic factors: the host, the pathogen, and the environment [15]. The relationship between these three factors can best be represented in the form of a triangle. It is the balance between these three components that decides the initiation and progress of disease. For initiating disease development, an interaction between a highly virulent pathogen and a susceptible host in a disease favourable environment is required. The environment plays a major role in modulating the virulence of the pathogen [16–18] as well as reducing the host defence [19] and thus increasing the susceptibility of the host. A pathogenic agent can certainly gain entry into the animal body and initiate disease development process but the immune system of host can phagocytise the pathogen (e.g., by secreting chemical factors) and thus check the disease progress. On contrary to this, the host can also influence the environment by alterations in the microclimate requirements for disease production for example, abrasions, wound, malnutrition, path physiological conditions, and immunocompromised status [20]. A thorough consideration of interactions amongst these factors allows assessment of risk for disease outbreaks and intervention to reduce the amount of disease.

The severity of onset of clinical disease in the host is decided chiefly by the pathogenicity of the prevalent population of the pathogen. The term pathogenicity includes both virulence and aggressiveness. The adaptation mechanisms of the pathogen to the altered environmental factors play an important role in determining its survival in the host and the environment as a whole. The reduction in heterozygosity in disease resistance genes of bighorn sheep (*O. canadensis*) populations has been associated with highest lungworm parasite loads [21] as compared to domestic sheep which with a lengthier period of local adaptation and enhanced vigor might have also conferred resistance to common parasitic diseases. *Muellerius* spp. infections also typically do not produce clinical disease in domestic sheep [22] but may be more pathogenic in nonadapted hosts such as bighorn [23, 24] and possibly mouflon [25].

The foremost host factor affecting disease development is the presence of susceptible animals in the population. If the host population is largely susceptible to the pathogen in the vicinity, the disease may have the privilege to get transformed into an epidemic. The key player in determining the susceptibility to any pathogen is the immune status of the animal which, in turn, relies on number of environmental variables for its fluctuations. The preceding immune status of the host is frequently critical in determining the occurrence of disease; for example, low virulence pathogens usually produce clinical disease only in immunocompromised hosts while highly virulent pathogens may show morbidity even in healthy host. Animals whose lungs are already compromised from previous diseases usually fall prey to toxicity by leukotoxins and lipopolysaccharides, both potent toxins that, in high levels, act as chemotactic factors for inflammatory cells and promote inflammation and severe lung damage [26]. In kids, such acute outbreaks can occur with low morbidity rates but high mortality rates.

While lungworm infestations in sheep are quite common, the severity of lung lesions was observed only in sheep regularly exposed to high concentrations of volcanic gases

after the eruption of Kīlauea in 2008 which may have contributed to immunocompromised lung health, reduced resistance to parasitic infections, and increased susceptibility for severe inflammatory reactions [8]. Such severity of disease is also observed in conjunction with bacterial and/or viral infections or other stress factors characteristic of bighorn sheep pneumonia complex [27–29].

4. Environmental Variables

Environmental variables have conventionally been accepted as the major determinants for disease development (Figure 1). Even the traditional and chemical disease prophylactic and therapeutic control measures employ this concept for manoeuvring the environment to make it less congenial for disease progress. The prevalence of lung disease is unevenly distributed over the world [30] and can be traced down to regional environmental challenges along with other factors such as nutrition. As it is difficult to assess the prevalence, duration, and amount of exposure, the precise risk each environment factor poses is hard to define. Wildlife species of European mouflon sheep (*Ovis gmelini musimon*) translocated to Hawaiian Islands for sport hunting provided a unique opportunity to understand how disease processes may be affected by environmental conditions [8].

5. Aerographic Conditions

The aerographic conditions commonly include the state of atmospheric air in terms of temperature, wind velocity, clouds, precipitation, and volcanic eruptions. The prevailing climatic conditions have a major impact on the survival of the pathogens [31]. An alteration in weather conditions of a geographical area has always witnessed an outburst of infectious diseases and has been labelled as predisposer of disease epidemics. Small ruminants are well adapted to extreme temperatures, with their body hair coats providing insulation against cold and heat [32]. Sheep, in general, are more susceptible than goats to high temperatures and humidity [33]. Any alteration in the environmental temperature affects the incubation period, the life cycle (the time between infection and sporulation), and the contagious period (the time during which the pathogen continues to propagate the infection amongst the population). At higher temperatures the life cycle of the pathogen usually gets speeded up with the result that epidemics develop at a faster rate. Under cooler conditions, the pathogens develop dormancy and the progress of epidemic is slower leading to a decline in incidence as well as severity of disease.

High humidity increases the risk of heat stress at any air temperature. The heat index (temperature + humidity) is considered as a more accurate measure of heat stress (hyperthermia) by veterinarians than temperature alone [34]. Heat stress lowers the natural immune defense of animals, thus, making them more susceptible to disease. An increase in the incidence of pneumonia is a common observation in extremely hot weather [35]. The resistance to parasitic and other opportunistic diseases is also reduced. *P. multocida* often exists as a commensal in the upper respiratory tracts of majority of livestock species and has also been identified as the most frequently isolated bacteria from pneumonic lung [36] but the importance of predisposing factors in the development of pneumonia can never be overestimated.

Moisture also influences outbreak of respiratory diseases caused by microorganisms like bacteria and fungi and nematodes [37]. The influence of rain splash and running water on dispersal of pathogen is also important for explosive nature of the disease [38]. Free water or the collision of raindrops facilitates the dissemination of many fungi and nearly all bacteria. It is a useful adaptation for a pathogen that facilitates dispersal and germination as well as establishment of infection in the host. Pathogens like fungi and nematodes require a latent period for germination of spores and setting up of infection in the host animal. As both these processes are time taking as well as unavoidable for disease initiation, the duration of persistence of favourable climatic conditions has an important influence on infection.

In addition, the dissemination and resulting concentration of the pollutant may vary significantly depending on the prevailing (e.g., meteorological) conditions at that time. Patterns of atmospheric dispersion, for example, change not only in relation to wind speed and direction but also temperature inversion effect and atmospheric stability [39]. Statistically significant relationship was found between incidence of pneumonia as a cause of lamb death and climatic factors such as rainfall, humidity, and intensity and direction of wind [40].

Animal housing is also an important consideration in evaluating the impact of outdoor aerographic conditions on the health of the animals. Animals living indoors are less likely to be affected by rain and thunderstorms but poor ventilation and unhygienic barns are usually associated with severe outbreaks of respiratory diseases. The grazing goats have been reported to show about 2-3-fold higher morbidity as compared to the stall-fed animals [41]. Amongst the indoor factors responsible for microbial pollution the most important is the animal itself and its bedding material. Confinement of circulating air also prevents dissemination of the microbial load and hence facilitates the disease initiation. The moisture content of the bedding material may further assist in production of spores and metabolites of different bacterial and fungal strains resulting in a chronic inflammatory and immunosuppressive response.

6. Climate

Climate is the statistical information that expresses the variation of weather at a given place for a specified interval of time. Climate change is likely to directly affect the physiological profile of animal by altering the homeostasis and other thermoregulatory functions and hence its health and productivity. Climate may also influence health of animals indirectly by disturbing the nutritional supply thus, decreasing resistance to diseases and pests.

Impact of Climate Change. Inter-Governmental Panel on Climate Change has projected that global earth temperature will increase by 1.8–4.0°C by the end of this century [42].

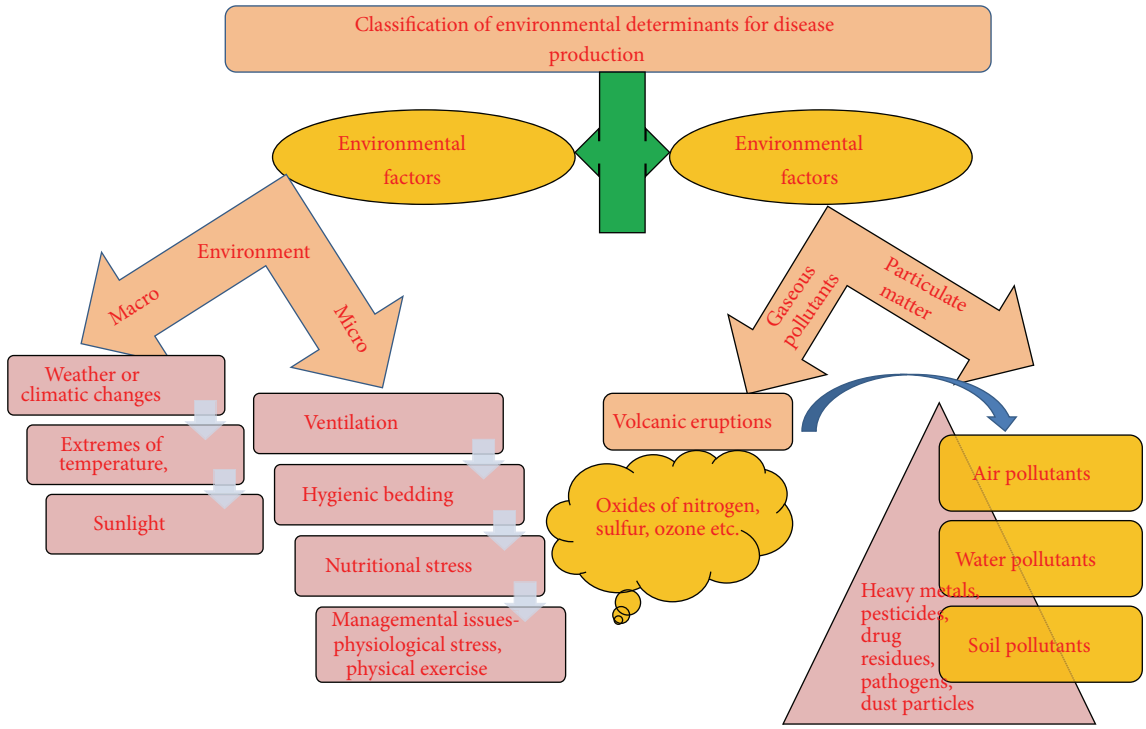

Figure 1: Classification of environmental determinants for disease production.

This increase in global temperature could potentially cause scarcity of water and food resources and dissemination of infectious diseases and heat-related deaths. The significance of temperature is further promoted in context of temperate regions as compared to the tropics, where temperatures are relatively uniform throughout the year [43]. Further, the subsequent climatic changes are expected to increase the possibility of vector-borne and other diseases and transformation in pattern of disease transmission. The maximum effect of climatic variation on transmission of disease is likely to occur at the lower and upper limits (14–18 and 35–40°C, resp.) of the range of temperature at which the transmission of infection takes place [44]. Rise in temperature and alterations in rainfall pattern will favor the disbursal of vector populations to unforeseen areas (higher altitude or temperate zones) [45]. In the tropics, diurnal oscillations in temperature are greater than the seasonal fluctuations, inducing many pathogens to sporulate by the combination of the decrease in temperature and the increase in humidity at night. The occurrence of Bluetongue in Europe and Rift Valley Fever in goats in East Africa are two well-documented examples of increased vector-borne disease risk in goats associated with climate change [46]. Further, microbial pathogens as well as their vectors may also show sensitivity to factors such as temperature, humidity, rainfall, ground water, wind velocity, and changes in vegetation and are bound to have an impact on emerging and reemerging infections of livestock. In a study conducted in Avikanagar (Rajasthan, India), cold stress along with frost and poor ventilation has been found to predispose lambs to *E. coli*-borne septicemia with major involvement of upper respiratory tract and lungs [47].

7. Atmospheric Pollution

Atmospheric pollution remains a major health hazard to all the living species throughout the world and shares about 8-9% of the total disease burden [7], but the risk is higher in developing countries, where poverty, lack of modern technology, and weak environmental legislation further substantiate the risk. The lungs serve as common interface between the animal body and the air environment in its close vicinity. Consequently, the lungs become a frequent dumping site for airborne pollutants. Thousands of environmental toxins and commercial chemicals such as heavy metals and pesticides are now in use, the particles of which may persist in the atmosphere as aerosol, fibres, fumes, mists, or dust. The effects of polluted air on domestic animals principally can either be caused by the indoor environment and by outdoor air pollution. Goats and, to a lesser extent, sheep are reared indoors but their indoor environment is quite comparable with the outdoor air conditions. Therefore, outdoor pollution is considered more important than the indoor pollution. Indoor pollution gains further significance in case of animals kept in overcrowded premises or in poor hygiene or ventilation.

7.1. Epidemiology of Atmospheric Pollution. Exposures to pollutants may occur through a number of pathways and exposure processes. Inhalation of environmental pollutants is generally over a considerable period of time and thus usually elicits health issues on chronic basis, but occasional inhalation of solid particles deposited from industrial exhaust on pasture land may directly cause an acute response. The increased incidence of pasture originated disease can be

attributed to their short stature due to which they breathe closer to the ground as compared to cattle and hence are more likely to inhale the solid particulates deposited on the pasture. The lesions produced in small ruminants such as sheep and goat due to air pollution are chiefly inflammatory in nature as was observed in 1952 smog disaster (London, UK) that increased respiratory tract hyperresponsiveness and ultimately resulted in respiratory distress (and right-sided heart failure) of cattle that were housed in the city [48] owing to high level of sulphur dioxide. Owing to high solubility sulphur dioxide mainly irritates the anterior air passage characterised by acute bronchiolitis and the accompanying emphysema.

7.2. Interplay between Atmospheric Pollution and Health. The relationship between pollution and health is both a multifaceted and conditional process. For pollutants to have an adverse effect on health, susceptible individuals must receive a minimal dose of the pollutant, or its metabolite, over a period sufficient to trigger detectable symptoms. Pollutants rarely occur in isolation; typically they exist in combination [7]. Exposures are therefore not singular rather a mixture of pollutants, often with varied origins, some of which may have additive or synergistic effects [49, 50]. Unravelling the effects of individual pollutants is a herculean challenge that has yet to be adequately resolved in many areas of environmental toxicology. Individual pollutants may be implicated in a wide range of health effects, whereas few diseases can directly be attributed to a single pollutant. Long latent intervals, cumulative exposures, and multiple exposures to different pollutants which might act synergistically all create difficulties in unravelling associations between environmental pollution and health. Health consequences of environmental pollution are thus unpredictable, even for pollutants that are inherently lethal; the ultimate outcome will depend on the coincidence of both the discharge and dispersion processes that determine the rate of appearance and dilution of the pollutant in the environment.

7.3. Mechanism of Atmospheric Pollutants. Irrespective of the origin, the ultimate health hazard imposed by all pollutants depends upon their persistence, mobility, biotransformation, and their toxicity profile. The problems associated with the release of persistent pollutants like chlorinated pesticide, DDT (Dichlorodiphenyltrichloroethane), into the environment were highlighted with recognition of the global extent of contamination and a wide-range of environmental and health effects [51]. The signature movement in this regard took long back in 1962 when an American biologist, Rachel Carson, published a book, Silent Spring, and resulted in a large public protest that eventually led to a ban on agricultural use of DDT in the USA in 1972. This book detailed the environmental impacts of the indiscriminate spraying of DDT in the USA and questioned the logic of releasing large amounts of chemicals into the environment without fully understanding their effects on ecology or human health. Similar stories are now around the world in respect to chlorofluorocarbons and other atmospheric pollutants that are accepted as greenhouse gases or scavengers of stratospheric ozone [52] and perhaps also endocrine disruptors [53].

7.4. Factors Affecting Pollutants Severity. Mere persistent nature of a pollutant does not endorse the health risk; its presence in a form that is accessible to the lungs is also important to produce respiratory disease. The development of environmentally induced lung disease is a function of the intensity and duration of the exposure as well as the inherent toxicity of the inhaled substance and susceptibility of the host. The physical status of the inhaled substance (solid, fume, or mixture), the particle size, and other physicochemical characteristics (like solubility) principally determine the initial location of disease development. Smaller particles (0.1 to 1.0 μ) are more likely to reach the lung alveoli, but airborne particles up to 5 microns in size may also do so. In general, larger particles (10 μ or greater) are trapped and removed by the mucus and cilia of the upper respiratory tract. Inorganic mercury is persistent but less toxic and less readily bioavailable than methyl mercury, which gets converted naturally through chemical reactions by microorganisms [54, 55]. Conversely, many solid wastes pose little risk as long as they remain in their original form. The problem arises when their decomposition takes place, either because the decomposition products are inherently more toxic or because they show an increased accessibility to the respiratory system.

Ventilation is often a managemental problem for indoor sheep and goat farming. High level of ammonia is a common finding in the indoor atmosphere of small ruminants. Ammonia is a highly hydrosoluble respiratory toxicant which causes chronic dyspnea and clinical pictures consistent with restrictive lung dysfunction, obstructive lung disease, and bronchial hyperreactivity [56].

7.5. Types of Atmospheric Pollutants. Dumping of waste materials of either chemical or biological origin represents a major source of air pollution, though final release into the wider environment may only occur when these materials decompose or break up.

7.5.1. Particulate Matter. Respirable particles of air pollutants and gaseous agents affect different parts of the respiratory tree depending upon their inherent characteristics [57]. For particulate pollutants, particle size is more important while for gasses, relative solubility is important. In a study conducted on Hawaii Island, higher incidence of pathological lesions has been documented in lungworm infested sheep that were exposed to gaseous emissions from Kīlauea Volcano in contrast to lungworm infested sheep not in vicinity of volcanic discharges though latter had significantly more upper respiratory tract inflammation and hyperplasia suggestive of chronic antigenic stimulation, possibly associated with exposure to fine airborne particulates owing to reduced plant ground cover during extended drought conditions [8].

7.5.2. Gaseous Pollutants. Probably, gasses from Kīlauea Volcano such as sulfur dioxide contributed to severity of respiratory disease principally associated with chronic lungworm

infections at Mauna Loa. Sulphur dioxide, because it is highly water soluble, initially affects the upper airway, while ozone, with its medium solubility, initially affects the middle airways and nitrogen dioxide, with its low solubility, initially affects the lower airways.

To affect the respiratory tree, the gaseous pollutants must be inhaled in a sufficient volume so that a minimal alveolar concentration is reached. Thereafter, the toxic potency of the pollutant will decide the degree of damage. Different physiological and environmental factors will also exert an influence on the overall toxicity; for example, physiological stress, metabolic acidosis, hypoxia, hypotension, hyponatremia, or hypomagnesaemia will potentiate the toxicity while CNS excitation or hypernatremia will subdue the hazard.

7.5.3. Microbial Contaminants. Bacterial infections in a sheep and goat farm are a common clinical and subclinical finding [58–60]. Some common respiratory commensal bacteria include *Pasteurella* spp. [36], *Staphylococcus* spp., *Streptococcus pneumoniae* [61], *Arcanobacterium pyogenes*, *Haemophilus* spp., and *Klebsiella pneumonia* while the common mycoplasmas isolated from sheep and goats are *Mycoplasma capricolum* subsp. *capripneumoniae* (a causal agent of caprine contagious pleuropneumonia), *M. mycoides* subsp. *capri* (involved in contagious agalactia syndrome), *M. bovis* [62], and *M. ovipneumoniae* [63]. Out of these, *M. ovipneumoniae* is one of the most important mycoplasmas involved in the respiratory diseases of sheep. Combined effects of ammonia and bacterial endotoxins predispose the animals to respiratory infections with viruses and bacteria, both primary pathogenic as well as opportunistic species. Although food producing animals appear to be capable of maintaining a high level of efficient growth in spite of marked degrees of respiratory disease [64], at a certain level of respiratory insufficiency rapid growth can no longer be attained. In that case the production results will be uneconomically. The viral infections also predispose the host to bacterial infection by a direct damage to respiratory clearance mechanisms and lung parenchyma, facilitating translocation of bacteria from the upper respiratory tract and establishment of infection in compromised lung and secondly, by interfering with the immune system's ability to respond to bacterial infection [65, 66].

8. Oxidative Stress as Predisposer

Respiratory diseases in sheep and goats are generally an outcome from physiological stress with viral and bacterial infections and adverse weather exposure [67]. Predisposing causes [68] are generally synergistic and include age, stress (comingling, weather, nutritional changes, etc.), and immunological background. Environmental risk factors include climate, ambient temperature, dust particles, stocking density, humidity, ventilation, and shipping distance.

Oxidative stress is a normal physiological phenomenon [69]. Under normal conditions, the physiologically important intracellular levels of reactive oxygen species (ROS) are maintained at a minimal requisite level by various enzyme systems participating in the *in vivo* redox homeostasis. Stress is one of the basic requirements for disease development (Figure 2) [69, 70].

It can have several origins like environmental extremes for example, cold, heat, hypoxia, physical exercise, or malnutrition. Stress can also be categorized on the basis of duration and onset as acute and chronic stress. The stress due to exposure of cold or heat is generally acute and temporary and is released with the removal of cause. Similarly stress due to physical exercises or complete immobilization [71] is also acute in nature but nutritional and environmental stresses usually persist for a longer period of time. Dust, transporting, weaning, handling, mingling with infected animals, overcrowding, dehorning, and castration all add to the onset of disease. Decreasing the number of stress factors associated with a disease is also an important step in prevention. The less an animal is exposed to the stress factors, the more likely it will maintain an integral immune system to defend itself against infectious organisms [72]. Oxidative stress resulting from persistent inflammation due to an inhaled irritant can be the major factor involved in the change of the dynamics of immune responses of the respiratory system. These alterations can create an immunological chaos that could lead to loss of architectural integrity of cells and tissues ultimately leading to chronic conditions or cancers [73, 74].

The significant contribution of predisposing factors in the development of pneumonic lung owing to commensal pasteurella infection is well known [36]. A primary infection with *Mycoplasma ovipneumoniae* is frequently isolated from pneumonic sheep, but it can also be found in the respiratory tracts of healthy animals [75]. Nevertheless, it may predispose sheep to invasion of the lower respiratory tract by other organisms such as the parainfluenza-3 virus and *Mannheimia haemolytica* [76, 77]. Few reports also implicate *Mycoplasma ovipneumoniae* as a cause of severe respiratory disease in goats [78, 79]. Occurrence of clinical respiratory disease due to these pathogens is associated with poor management practices and occur as a consequence of severe stress for example, transportation stress, viral infections (e.g., parainfluenza-3 virus), lung parasites, prior bacterial infections, overcrowded pens, poor housing conditions, sudden environmental changes, and other stressful conditions.

9. Prophylactic and Therapeutic Management

The first step in preventing environmentally related lung disease is to recognize the exposure-disease relationship. Then, primary prevention may be accomplished with a reduction, modification, or elimination of the exposure or environment. Other interventions require global approach to prioritize and target environmental modifications with public health policy implications. Educating about the ill effects of air pollution is also an important aspect of prevention of environmentally induced lung disease.

Broad spectrum antibacterial agents may be effective in treating bacterial infections in sheep and goats and may include fluoroquinolones such as enrofloxacin, ciprofloxacin, florfenicol, and ceftiofur along with suitable anti-inflammatory agents [80–83]. While selecting the drug

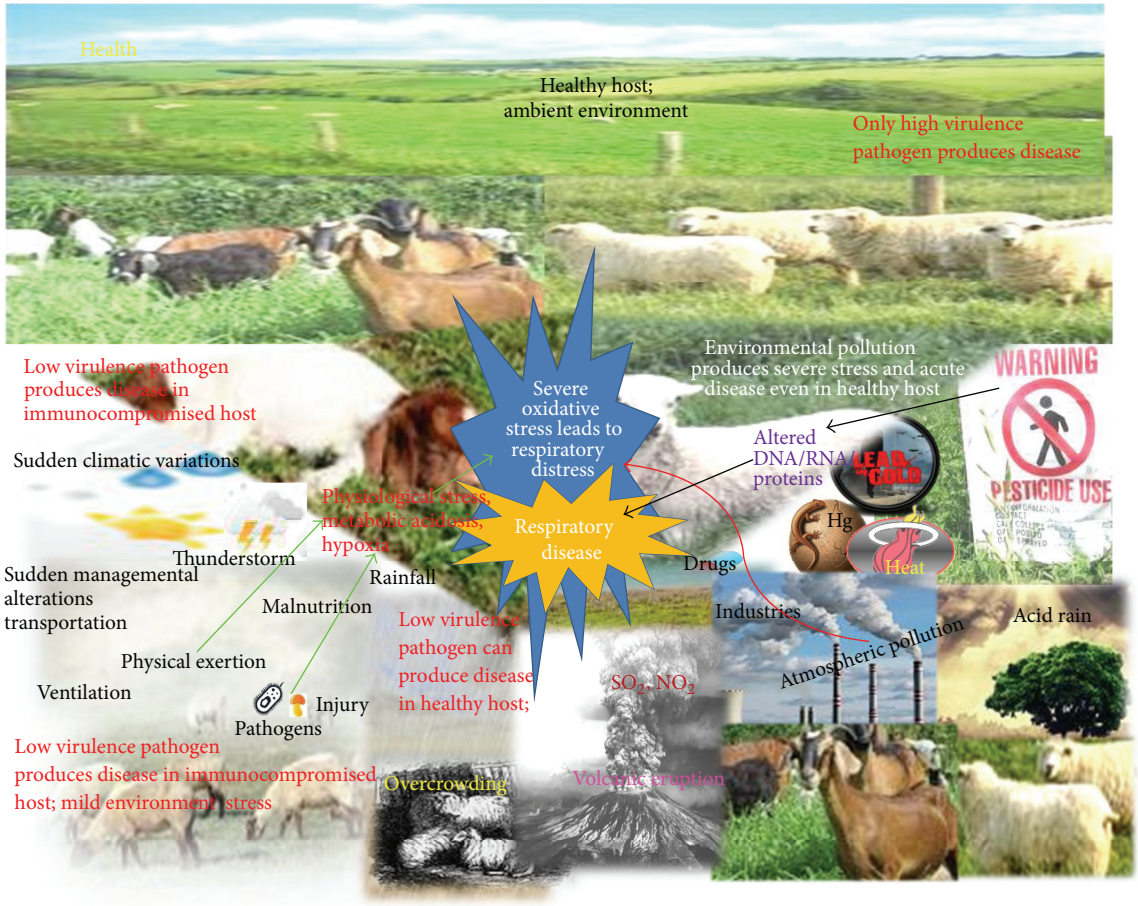

Figure 2: Environmental attributes to oxidative stress leading to initiation and progress of respiratory diseases.

combinations and their respective dosage regimen, drug interaction should to be considered in view of the pathophysiological status of the animal [84]. Several natural feed components have received great attention in the last two decades, and several biological activities showing promising anti-inflammatory, antioxidant, and antiapoptotic-modulatory potential have been identified [85–87]. Plants such as *Ocimum sanctum* have been used for ages to prevent and cure viral infection of man and animals [88].

Interleukin-1beta (IL-1beta) and tumor necrosis factor-alpha (TNF-alpha) have been proven to mediate the development of numerous inflammatory lung diseases [74]. A number of common indigenous plants such as *Cimicifuga racemosa*, *Mimosa pudica*, and so forth have shown excellent anti-inflammatory potential and can be added to regular feeding schedule of small ruminants for prophylaxis [86]. Zinc supplementation has been found to shorten duration of severe pneumonia in human infants. Perhaps, zinc as an adjuvant hastens recovery and reduces antimicrobial resistance [89]. Antioxidant supplements also seem to modulate the impact of ozone and particulates pollutants on lung function [90]. Vitamin C and E may blunt effect of ozone on lung function but do not seem to prevent symptoms.

10. Conclusions

Although the disease development can be described as three sets of two-way communications (pathogen-environment, host-environment, and host-pathogen), this is a generalization. All three groups of factors interact in a highly variable manner in any real life scenario, often in nonlinear ways that are difficult to compute and forecast.

Estimating the contribution of environmental pollution to the burden of disease is far from simple. The global atmospheric pollution scenario is too difficult to classify and define completely. Moreover, it is never static; new pollutants are being introduced to the air every day and too little is known about their interactions with respiratory health, or about their levels of exposure, to make reliable toxicity appraisal. These difficulties are more pronounced in developed countries, where disease surveillance, reporting of mortality, environmental monitoring, and population data systems are all relatively well approved. Still precise evaluation of the disease burden is yet worth the endeavour.

The animal biodiversity available in our country is a virtual goldmine of germplasm. Some of the indigenous breed of livestock like Jamunapari goat have unique characteristics of

adaptability to adverse agroclimatic conditions, better disease tolerance, feed conversion efficiency, and zero managemental requirements. Therefore, maintain a livestock population that is sustainable in the present everday changing climatic scenario is a challenging task, which would require a change in breeding policy, perpetuating disease resistant and climate adaptable traits, capacity building, and regional and global cooperation.

Conflict of Interests

The authors declare that there is no conflict of interests in publication of this work.

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