Original Article

Detection of Contagious Caprine Pleuropneumonia in Three Provinces of Iran: 2017-2018

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How to cite this article: **Esmaeili H, Joghataei SM. Detection of Contagious Caprine Pleuropneumonia in Three Provinces of Iran: 2017-2018. Archives of Razi Institute. 2024;79(6):1263-1270.** DOI: 10.32592/ARI.2024.79.6.1263

Article Info:
Received: 26 February 2024
Accepted: 15 April 2024
Published: 31 December 2024

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ABSTRACT

Contagious caprine pleuropneumonia (CCPP), a highly contagious mycoplasmal disease, represents a significant threat to goat populations due to its rapid transmission. Despite the existence of CCPP cases in Iran, there has been a paucity of research conducted on this disease. To address this knowledge gap, the present cross-sectional study focused on the reporting and investigation of CCPP cases, along with the clinical signs and necropsy findings of this disease, in Tehran, Kermanshah, and Yazd provinces. An investigation was conducted into a CCPP outbreak affecting 4,400 goats on six breeding farms. The study encompassed both male and female goats of imported breeds, including Alpine (1,500), Saanen (1,700), and Murcia Granada (1,200), across a range of age groups. The clinical signs exhibited by the infected animals were meticulously observed and recorded, and comprehensive necropsy observations were documented. A variety of samples were obtained for polymerase chain reaction (PCR) testing to confirm the presence of caprine pleuropneumonia virus (CPV), including nasal swabs (35), pleural fluid (65), and lung tissue (83). A total of 516 goats (11.7%) were diagnosed with CCPP, and among them, 287 goats (6.5%) unfortunately succumbed to the disease. The morbidity rates in Tehran, Kermanshah, and Yazd provinces were 11.7%, 12.7%, and 10.5%, respectively, while the corresponding mortality rates were 6.5%, 7.2%, and 5.7%. The principal clinical indications were fever (89%), anorexia (68%), nasal discharge (78%), dyspnoea (54%), painful coughing (96%), and grunting (84%). During necropsy examinations, the primary post-mortem signs observed were lung consolidation and hepatization (98.3%), the presence of extensive straw-coloured serous fluid and fibrinous exudate on the pleural surface (94%), alveolar cellular exudate (90.6%), and adhesion of lungs (46%). The clinical and necropsy findings were entirely consistent with a diagnosis of CCPP. Verification of the disease agent's existence was achieved in 183 cases (63.7%) based on the results of the polymerase chain reaction (PCR) test. It is evident that there is a paucity of clinical and well-documented studies on CCPP disease in Iran. However, the findings of this study contribute to the expansion of our knowledge of CCPP and serve to raise awareness of its presence among Iranian goats. It is strongly recommended that vaccination be included as an essential measure for the prevention of CCPP.

Keywords: Contagious Caprine Pleuropneumonia, *Mycoplasma Capricolum*, Goat.

1. Introduction

According to historical records, sheep and goats were domesticated in various regions of Iran, with the West Asia region being a major center for this livestock. Initially, there were more domesticated goats than sheep. To date, Iran ranks among the top countries in terms of sheep and goat populations worldwide. Various infectious diseases, including pneumonia, pose challenges to the productivity of goats. Pneumonia is particularly remarkable as it can cause mortality and reduce productivity. Among the different causes of pneumonia, the agent responsible for contagious caprine pleuropneumonia (CCPP) holds notable importance and has been present among Iranian goats for an extended period (1-3). CCPP is a highly contagious and rapidly infectious mycoplasmal disease that affects the majority of goat flocks. In light of the considerable impact of this illness, there is a global initiative to gain a deeper understanding of it, with the aim of enhancing its detection, avoidance and management. (4, 5). It has been postulated that in the distant past, Iranian goat breeders employed a method whereby the lungs of deceased animals, which were presumably afflicted with the same disease, were crushed and the resulting paste was affixed to threads. Subsequently, the goats' ears were sewn with these special threads, which were used for autologous vaccinations and the prevention of further casualties. CCPP, caused by Mycoplasma capricolum subspecies capripneumoniae (Mccp), is transmitted via respiratory droplets inhaled during close contact. The infection is disseminated throughout the flock by a carrier or infected animal. The presence of anorexia, fever, and respiratory symptoms, including coughing, grunting, inability to move, standing with front legs widely separated, a rigid and lengthened neck, constant salivation, and nostril discharges, indicate that the disease impacts the respiratory system. This disease has a high morbidity rate (approximately 100%) and mortality rate (80-100%). It presents specific necropsy signs, including the formation of straw-coloured pleural fluid, extensive lung hepatisation and pleurisy due to fibrinous pleuropneumonia. A number of variables may contribute to the clinical progression of CCPP, including immunosuppression, co-existing viral infection, large flock size, advanced age, rapid climate change, and stress (2-4). In 2020, a study conducted by Abd-Elrahman et al. in one of Egypt's provinces examined 200 goats for CCPP, resulting in 30 deaths. The clinical signs observed included a fever reaching 41°C, copious nasal discharge, dyspnoea, corneal opacity, and labored respiration. Additionally, post-mortem findings include severe lung marbling, straw-coloured exudate within the thoracic cavity, thickening of interlobular septa, and adhesion between the lungs and pleura (6). In a study conducted by Hussain et al. in Pakistan, which examined CCPP in Beetal goats, the most frequently observed clinical signs included severe coughing, dyspnoea, purulent nasal secretion, increased respiration rate, emaciation, and pyrexia. Additionally, necropsy revealed extensive consolidation of the lungs, followed by alveolar exudation and pleural adhesion. The findings of this study indicate that CCPP is a highly prevalent condition in this breed under subtropical conditions, with a notable impact on mortality rates. Global initiatives are currently being implemented with the objective of combating this novel and significant threat to goat farming on a global scale (4). In Iran, despite the presence of this disease among goats of different breeds, this disease has been less studied. Additionally, the clinical indicators, which are often non-pathognomonic and mistaken for other respiratory illnesses, are linked to the pathogenesis of the lower respiratory tract. Consequently, there is a need for a deeper understanding and recognition of the clinical signs and post-mortem lesions to implement effective preventive measures. To this end, the present study was conducted to determine the incidence of CCPP and gain a more comprehensive understanding of the clinical manifestations and necropsy findings in goats imported into Iran in some provinces.

2. Materials and Methods

2.1. Animals, Herd Conditions, and Study Area

The study was conducted in Tehran, Kermanshah, and Yazd provinces between January 2017 and January 2018 (Figure 1). The study population comprised 4,400 male and female goats of various ages. With regard to the breed, the goats included in the study comprised Saanen and Alpine breeds imported from France, as well as the Murcia Granada breed imported from Spain. A total of 1,700 Saanen and 1,500 Alpine goats were observed in Tehran and Kermanshah provinces, respectively. A total of 1,200 Murcia Granada goats were present in the Yazd province. The goats were reared under intensive farming conditions. The study was conducted on six breeding farms, four of which bred Saanen and Alpine breeds, and two of which bred the Murcia Granada breed. The goats on these farms received regular vaccinations in accordance with the guidelines set out by the Iranian Veterinary Organisation. The vaccines administered included those for PPR, enterotoxemia, Rev 1, goat-pox, and FMD. However, the herds did not receive a vaccine against CCPP disease.

2.2. Necropsy and Sampling

During the specified time frame, suspected cases of CCPP were observed in the six aforementioned breeding farms, where they were separated for further investigation. The animals displayed a range of symptoms, including a persistent cough, fever (40.5–41.5°C), nasal discharge, respiratory and oral breathing difficulties, lethargy, weight loss, and cessation of milk production and nestling. The animals displaying clinical indications suggestive of CCPP were identified as potentially infected and subjected to close monitoring as part of the study. The animals were observed meticulously in order to monitor the progression of the disease and gather data on the clinical manifestations. To investigate the outbreak, nasal swabs were collected from 35 goats displaying signs suggestive of CCPP after proper cleansing of the external nares. Nasal swabs were taken

from 15 animals that did not display clinical signs and were therefore used as a control group. Furthermore, necropsies were conducted on 286 deceased goats within four hours of their demise (Table 1). The results of the necropsies were duly recorded. The animals were either humanely euthanised on the farms or died naturally. Molecular confirmation sampling entailed the collection of pleural fluid samples from 65 goats and lung tissue samples from 83 goats. The pleural fluid was obtained using sterile syringes, while the lung tissue was collected from the interface between consolidated and unconsolidated areas. The samples were placed in an icebox and promptly dispatched to the laboratory for analysis. Upon arrival at the laboratory, the samples were stored at -20°C until analysis.

2.3. DNA Extraction

The DNA extraction was conducted in accordance with the instructions provided by the manufacturer of the HiPurATM Multi-Sample DNA Purification kit (HiMedia, Hungary). The concentration of DNA in each extraction was determined using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific Inc., USA). The extracted DNA was diluted in the elution buffer provided in the DNA extraction kit to achieve a final concentration of 10 ng/µl, based on the measured concentrations.

2.4. PCR Amplification

To confirm the presence of Mccp, a polymerase chain reaction (PCR) was conducted using the method described by Woubit *et al*. This method is a conventional molecular technique (8, 9). The PCR reactions were performed using a Bio-Rad T100 thermal cycler. The particular amplification procedure was conducted in a final volume of 50 microlitres. The reaction mixture comprised 34 µl of distilled water, 0.5 µl of dNTPs (at concentrations of 150 µM for dCTP and dGTP, and 300 µM for dATP and dTTP), 3 µl of $MgCl_2$ (at a The concentration of the solution was 1.5 mM, and 5 µl of 10× Taq Buffer (Qiagen) was also included. Additionally, 1 µl of each primer (0.4 µM) was added (Mccp-spe-F: 5′-ATCATTTTTAATCCCTTCAAG-3′ and Mccp-spe-R: 5′-TACTATGAGTAATTATAATATATGCAA-3′). The amplification of a DNA fragment of 316 bp (ArcD gene of Mccp) was achieved using the following reagents: 5 µl of the sample, 0.5 µl of Taq polymerase (1 unit, Qiagen), and 5'-TACTATGAGTAATTATAATATATGCAA-3' (spe-R). The PCR cycling conditions comprised an initial denaturation step of two minutes at 94 °C, followed by 35 cycles of 30 seconds at 94 °C, 15 seconds at 47 °C, and 15 seconds at 72 °C. Subsequently, a final extension step of five minutes at 72 °C was conducted. Thereafter, the PCR products were subjected to electrophoresis on a 1.5% agarose gel, which was run for approximately two hours at 80 V. The use of a UV transilluminator enabled the observation and documentation of the magnified fragments.

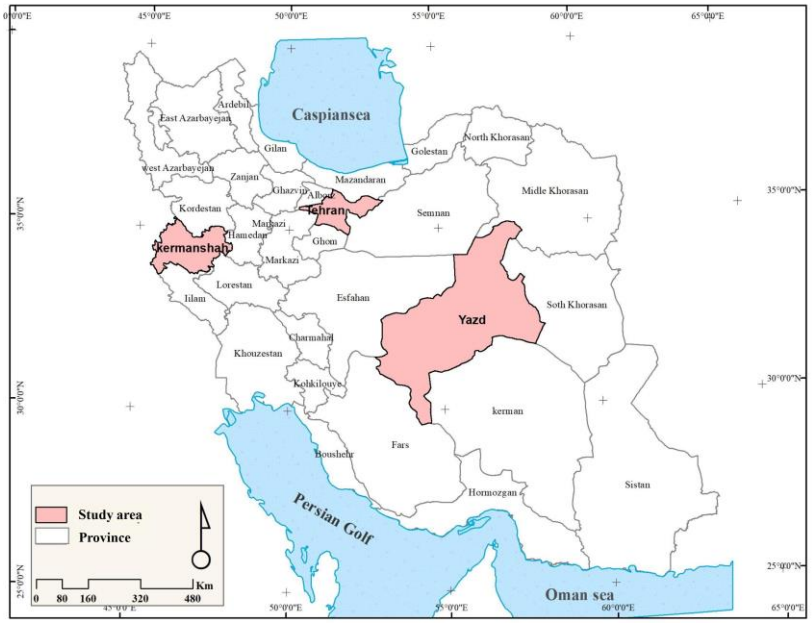

Figure 1. Map depicting the provinces that were examined in the present study.

Table 1. Morbidity and mortality rates in farms by province.

Location	NO. of Farms	Breed	Total Animals	Morbidity		Mortality	
				No.	%	No.	%
Tehran	2	Saanen and Alpine	1750	205	11.7	115	6.5
Kermanshah	2	Saanen and Alpine	1450	185	12.7	104	7.2
Yazd	2	Murcia Granada	1200	126	10.5	68	5.7

2.5. Data Analysis

The data gathered from both field and laboratory settings were meticulously documented and structured in a Microsoft Excel spreadsheet. A comprehensive screening process was employed to ensure accurate coding and the identification of potential errors. Subsequently, the data were subjected to statistical analysis using STATA 13.0 statistical software. The morbidity and mortality rates were calculated in order to assess the occurrence of the disease and to measure the strength of the association, respectively. The data were subjected to a statistical analysis, specifically a chi-square test. A significance level of $P<0.05$ was employed as the threshold for determining statistical significance (10, 11). The utilisation of these analytical methods was employed with the objective of gaining insights into the disease outbreak and identifying potential relationships and associations between variables.

3. Results

3.1. Clinical Findings, Morbidity, and Mortality

A total of 286 CCPP-related deaths were recorded during the course of the present study. The disease morbidity in Tehran, Kermanshah, and Yazd was 11.7%, 12.7%, and 10.5%, respectively, while the mortality rates were 6.5%, 7.2%, and 5.7%, respectively (Table 1). No significant difference was observed between the age of morbidity and mortality among the observed cases (Table 2). Similarly, no significant difference was noted between breed and mortality or morbidity rate ($p<0.05$). Furthermore, no significant difference was identified in the incidence of CCPP by region ($p<0.05$). Notably, in Yazd province, as the outbreak did not occur during the kidding season of Murcia Granada goats, there were no cases involving kids. A total of 4,400 goats from six herds were examined, and 516 (11.7%) exhibited clinical signs of CCPP. Of these, 287 (6.5%) died, resulting in a case fatality rate of 55.6%. In the majority of cases, death occurred within a period of 24 to 96 hours; however, some cases demonstrated recovery. Further observations revealed the presence of both acute and pre-acute forms of the disease. The clinical signs exhibited were largely similar across cases, with some minor variations. The most prevalent clinical signs observed included fever (40.5–41.5°C) (89%), anorexia (68%), depression (45%), reluctance to walk (26%), severe respiratory distress manifested as dyspnea (63%), fast breathing (56%), mouth breathing (56%), nasal discharge (78%), and sudden death (36%). These were preceded by the appearance of sero-fibrinous, straw-coloured exudate from the nares, which later became thick, mucoid or purulent and rust-coloured. Additionally, the animals displayed difficulty in breathing (54%), wide separation of the forelimbs (66%), frequent painful coughing (96%) and grunting (84%). In most cases, severe respiratory signs appeared after a fever for approximately two days. The observed clinical signs were all confirmed to be associated with CCPP.

3.2. Gross Pathology

In all cases, the post-mortem lesions were limited to the thoracic cavity, which is suggestive of CCPP. The gross pathological lesions included consolidation of the lungs and hepatisation of entire lobes, often unilateral (98.3%) (Figure 2), as well as sero-fibrinous pleurisy, which was characterised by a fibrinous exudate on the pleural surface and an exudative effusion of straw-coloured serous fluid into the pleural cavity (in 94% of cases) (Figure 3). Additionally, there was extensive bronchoalveolar cellular exudate. The most common lesions were pulmonary consolidation (90.6%), pleural adhesion (72.8%), large mediastinal lymph nodes and a hard consistency (57.2%), fibrous adhesion of lungs to the thoracic wall (46%), granular or variegated lungs with red, yellow, white, and gray foci (33.2%), characteristic right unilateral lung enlargement (26.4%), and frothy discharge in the trachea (24%).

3.3. Mccp Detection and Confirmation Using PCR

Polymerase chain reaction (PCR) analysis demonstrated that a 316 base pair (bp) fragment of Mccp was specifically amplified in diverse sample types (Figure 4). Of the samples tested, 35 nasal swabs, 65 pleural fluids, and 83 lung tissue samples exhibited the presence of the 316 bp fragment. Furthermore, all 39 goats that tested positive for Mccp in their pleural fluids also exhibited positive results in their lung tissues. In contrast, none of the 15 control nasal swab samples displayed the presence of the 316 bp fragment. These findings indicate that a total of 183 cases (63.7%) tested positive for CCPP based on PCR analysis.

Table 2. Mortality rate by age and breed.

Age (months)	Number of Goats			Mortality		
	Saanen	Alpine	Murcia Granada	Saanen	Alpine	Murcia Granada
<12	430	370	140	25 (5.8%)	21 (5.6%)	0
12-36	860	790	955	52 (6%)	65 (8.2%)	56 (5.8%)
>36	410	340	105	31 (7.5 %)	25 (7.3%)	12 (11.4%)
Total	1700	1500	1200	108 (6.3%)	111 (7.4%)	68 (5.7%)

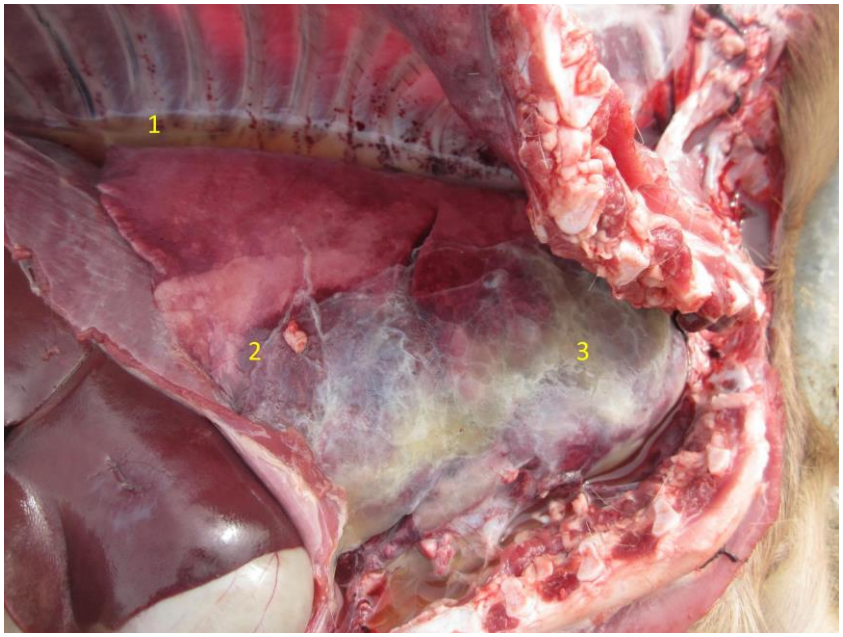

Figure 2. Post-mortem findings of Alpine goat that died of CCPP. Accumulation of straw-colored fluid within the thoracic cavity (1) and congestion and red hepatization lung (2). Note lung fibrin coat (3).

Figure 3. Gross lesions of a CCPP infected goat exhibiting extensive straw-colored pleural fluids (1) and sero-fibrinous pleurisy characterized by a thick yellow fibrin coat (2).

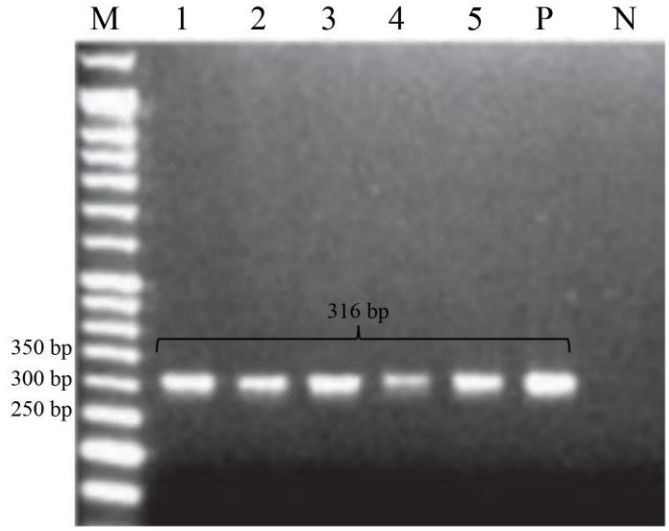

Figure 4. Agarose gel electrophoresis image showing specific amplification of 316 bp of Mccp. 1 and 2, nasal swab samples; 3 and 4, pleural fluid samples; 5, lung tissue samples; M, DNA ladder 50 bp; P, positive control; N, negative control.

4. Discussion

In light of the limited progress made in researching the devastating CCPP disease in Iran, the primary objective of the present study was to enhance the prognosis by investigating an outbreak of this disease among 4,400 goats. An accurate diagnosis of CCPP is of paramount importance, as it directly influences the selection of preventive and therapeutic measures and aids in developing effective control strategies (4). The study documented the clinical signs and necropsy findings observed in cases of CCPP among imported goats in Iran. It is noteworthy that the countries from which these animals originate also experience cases of CCPP disease (12). The results recorded in the current study will enhance the detection and diagnosis within the country's goat breeding industry. Failure to acknowledge the presence of this highly contagious disease in Iran could result in considerable economic losses for the goat breeding sector. A previous report from the World Organisation for Animal Health (WOAH) has documented the occurrence of CCPP in Iran. The report indicates that between 2006 and 2007, a total of 478 outbreaks of CCPP were reported in the country, affecting approximately 16,000 goats. These figures illustrate the considerable impact of the disease on the goat population in Iran during that particular period (13). It is noteworthy that, despite the existence of a World Organisation for Animal Health (WOAH) report, there is currently no documented evidence regarding the occurrence of CCPP in goat farms in Iran. Moreover, there is a dearth of epidemiological data pertaining to CCPP within the country. Previous studies have primarily concentrated on the detection of the causative agent in slaughterhouses. However, such studies are unable to provide insights into the disease's manifestation at the farm level or contribute to an understanding of the clinical signs and necropsy findings specific to CCPP in Iran. The most recent attempt to identify MCCP and CCPP in Iran, conducted by Namazi et al., focused on animals sent to slaughterhouses but was unsuccessful in PCR (14). In a further study, Khodakaram-Tafti et al. also reported the identification of Mccp with pneumonia from the lungs of slaughtered goats. The study focused on samples obtained from the slaughterhouse. Of the 2000 goats examined, three were found to be positive for Mccp. The molecular detection method and primers employed in a research study by Khodakaram-Tafti et al. were consistent with those used in the present study. The diagnosis of CCPP is mainly based on the examination of clinical signs of affected animals and post-mortem findings (3, 9). The present study aimed to provide a comprehensive overview of clinical observations and post-mortem findings to increase the knowledge of CCPP in Iran. The most common clinical indicators observed in affected goats included high fever, coughing and grunting, nasal discharge and respiratory distress. The predominant post-mortem findings included the presence of sero-fibrinous pleurisy, extensive straw-coloured serous fluid, pulmonary consolidation and hepatisation. All of the clinical signs observed were indicative of CCPP disease (3,4,6,9), which was confirmed by the molecular test results in these goats at a rate of 63.7%. Statistical analysis showed a significant correlation between the accuracy of molecular diagnosis and clinical examination results. Interestingly, a study by Dhaygude et al. in the state of Maharashtra, India, investigated cases of CCPP and reported clinical signs and post-mortem findings similar to those observed in the present study. The researchers also used a similar PCR method to confirm the presence of Mccp. The similarity in clinical and post-mortem findings between the two studies further supports the consistency and reliability of these observations in the diagnosis of CCPP (16). A study conducted by Tshume et al. in the Burana region of Ethiopia between November 2016 and April 2017 investigated CCPP cases. The clinical signs documented in the affected goats and the necropsy lesions observed were found to be consistent with the findings of the present study. The researchers employed a similar molecular methodology to that used in the present study to confirm the presence of Mccp in samples obtained from lung tissue and pleural fluid (11). In a study conducted by Saeed et al. in the Qassim region of Saudi Arabia between 2016 and 2017, cases of CCPP were investigated. The results of the aforementioned study were found to be consistent with those of the present study in regard to both clinical signs and necropsy findings. Similarly, as in the present study, sample collection was conducted and the PCR method was employed for molecular confirmation of the presence of Mccp (9). The current study did not identify a statistically significant correlation between the incidence of CCPP and the age of the goats. This finding is in accordance with the results reported in other studies. Similarly, other studies have also reported no significant correlation between age and the occurrence of CCPP. These include the studies by Shah et al. (2017), Nicholas (2002), Eshetu et al. (2007), Gizawu et al. (2009), and Hadush et al. (2009). These collective findings suggest that age may not be a determining factor in the susceptibility of goats to CCPP (11, 17). This study acknowledges and builds upon the previous chronological reviews conducted by various researchers and organizations, including Rurangirwa *et al*. (1987), Thiaucourt et al. (1992, 1996), Kusiluka (2002), Nicholas and Churchward (2012), Thiaucourt and Bolske (1996), Samiullah (2013), Prats-van der Ham *et al*. (2015), Yatoo et al. (2018), AU-IBAR (2013), Asmare *et al*. (2016), WOAH (2017), and EFSA AHAW Panel *et al*. (2017). These reviews have recorded similar clinical and necropsy findings, consistent with the present study's findings. The cumulative insights from these reviews indicate a changing trend in the understanding of CCPP, necessitating the development of future strategies. Initially reviews provided a general disease perspective, including its status, diagnosis, treatment, and prevention. However, recent reviews have shifted their focus towards novel diagnostic and preventive measures against CCPP. The aforementioned steps are designed to reduce the

likelihood of antibiotic resistance and eradicate the disease carrier state, while simultaneously supporting global initiatives aimed at preventing and controlling the disease. The findings of this study, which are in alignment with those of earlier evaluations, underscore the imperative for continued research and the development of efficacious countermeasures against CCPP. As emphasised in recent reviews, the advancement of diagnostic and prophylactic measures offers considerable promise for the prevention and control of CCPP on a global scale, as well as the reduction of antimicrobial resistance (4,12,18-21). The present study offers additional evidence in support of the presence of CCPP in the goat population of Iran, thereby corroborating the information presented in the previous WOAH report. The present study revealed a significant impact of CCPP, with 287 deaths attributed to the disease across three provinces in Iran and the three imported goat breeds. This finding underscores the severity of CCPP and highlights the pressing need for proactive planning and policy-making to control its spread effectively. The high mortality rate observed in the study clearly indicates the urgency in implementing measures to mitigate the impact of CCPP and prevent further losses within the goat population (4, 21). It is important to acknowledge that animals that have recovered from clinical disease may still act as carriers and potentially transmit the disease to other herds (4, 5). In Iran, it is customary for farmers to retain animals that have survived the infection. Furthermore, it is crucial to acknowledge that animals that survive the CCPP epidemic may still experience long-term consequences. As a consequence of the damage to their lung tissue and increased susceptibility, these animals are more prone to rapid reinfection with the same bacteria in subsequent lung diseases or infections. Consequently, their health declines at a more rapid rate, increasing the probability of mortality (3, 22). It is therefore imperative that a vaccination program is implemented in conjunction with a comprehensive prevention strategy, which should be coordinated by the country's veterinary organization, in order to effectively combat the CCPP disease (5, 23). Vaccination has been demonstrated to be an efficacious preventive measure against caprine circovirus papillomatous pneumonia (CCPP) caused by McCp, resulting in favourable outcomes with regard to the immunity of goats against the disease. A successful vaccination campaign in Kenya involved the administration of an inactivated F38 vaccine to 10,000 goats. Within three weeks of vaccination, there were no reported losses due to CCPP, and none of the 400 goats that were closely monitored displayed any clinical signs of CCPP over the subsequent six-month period (5, 24-26). These findings emphasise the efficacy of vaccination in preventing CCPP and its potential to significantly reduce economic losses associated with morbidity, mortality and decreased production. Furthermore, vaccination has the additional benefit of reducing the costs associated with treatment within the goat farming industry. In light of the findings of the present investigation, which highlight the significant prevalence of CCPP in Iran and its potential to inflict substantial economic losses, integrating a vaccination programme against CCPP into the national vaccination programme, overseen by the country's veterinary organisation, would not only serve to protect against financial losses but also represent a pivotal step towards safeguarding the country's goat industry and preventing trade restrictions and transportation disruptions related to the disease (5, 23).

Acknowledgment
The authors would like to express their gratitude and appreciation to all the ranchers and laboratory personnel who were involved in the study.

Authors' Contribution
Study concept and design: H. E.
Acquisition of data: H. E. and S. M. J.
Analysis and interpretation of data: S. M. J.
Drafting of the manuscript: S. M. J.
Critical revision of the manuscript for important intellectual content: H. E.
Administrative, technical, and material support: H. E.

Ethics
The authors of this study confirm that all ethical standards were adhered to in the preparation of the submitted article.

Conflict of Interest
The authors declare that they have no conflict of interest.

Data Availability
The data generated and/or analysed during the current study are available from the corresponding author upon request.

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