

Contagious Agalactia In Sheep And Goats: Current Perspectives

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Abstract: Contagious agalactia (CA) is a disease caused equally by four *Mycoplasma* species, in single or mixed infections. Clinical signs are multiple, including mastitis, arthritis, keratoconjunctivitis, pneumonia, and septicemia, non-specific, and expressed differently depending whether sheep or goats are affected, on causative mycoplasmas as well as type of husbandry. CA has been reported worldwide and its geographic distribution maps to that of small ruminant breeding areas. However, as current diagnostic tests are expensive and difficult to implement, it is certainly underdiagnosed and prevalence data are only available for a few countries. CA control relies on vaccines, chemotherapy and good herd management practices. It requires long-term commitment but is often unsuccessful, with frequent clinical relapses. The persistence of the etiological agents, despite their overall susceptibility to antimicrobials, comes from their genetic plasticity and capacity to escape the host immune response. The existence of asymptomatic carriers and the numerous sources of infections contribute to rapid spread of the disease and complicate the control and prevention efforts. Here we review all these aspects in order to highlight recent progress made and identify gaps in knowledge or tools needed for better disease management. Discussion also underlines the detrimental effect of contagious agalactia on small ruminant welfare.

Keywords: contagious agalactia, mycoplasma, disease prevention and control, diagnosis, pathogenicity and infection course, epidemiology

Introduction

Known for more than 200 years, contagious agalactia (CA) of sheep and goats was first reported back in 1816 in Italy, where it was quickly dubbed “mal di sito” (“disease of the place”) in reference to its ability to persist in an environment and contaminate newly introduced herds.¹ Its contagious nature resulted in the official designation of the name CA in 1871 by Brusasco.² Despite being termed “agalactia”, which refers to a marked drop or even a complete loss of milk production, its clinical outcome is not restricted to lactating females nor to only the udder/mammary glands. CA has multiple clinical signs that are often gathered under the acronym MAKEPS, for mastitis, arthritis, keratoconjunctivitis, pneumonia, and septicemia.³ It primarily results in a drop in milk production, followed by an increased general morbidity and mortality. It should be considered a serious threat to animal welfare in its acute phase as well as in its chronic form.

Mycoplasma (M.) agalactiae (Ma), the “historical” etiological agent, was successfully isolated for the first time in 1923 and characterized as “filterable but not invisible”, similarly to the agent of bovine pleuropneumonia.⁴ At that time, although the *Mycoplasma* genus was not yet established, this type of bacteria was already known to

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be parasitic and to cause chronic and generally difficult-to-eradicate diseases. *Ma* is still the main etiological agent in sheep, while three other (sub)species, namely *M. mycoides* subsp. *capri* (*Mmc*), *M. capricolum* subsp. *capricolum* (*Mcc*), and *M. putrefaciens* (*Mp*), are considered equally causative agents in goats, as their infection results in a similar clinical picture (Conclusion of the EC COST action 826).⁵ These three (sub)species are phylogenetically distant from *Ma* and belong or are close to a phylogenetically homogeneous group named the *M. mycoides* cluster.^{6–8} They share many genetic and antigenic traits making it more complicated to specifically segregate and identify them.^{7–10} Furthermore, mixed mycoplasmal infections in goats are regularly reported,^{11–13} which could complicate diagnosis and control measures. As clinical signs are non-specific, a CA outbreak cannot be confirmed without laboratory testing. These difficulties have prompted a huge field of research and development for diagnosis and control tools over the past years.^{14–17} However, the situation today is still not fully satisfactory and CA remains a significant burden in countries where small ruminant dairy production is important. Causative agents of CA are considered non-zoonotic, despite a report in 2014 of one case of human *Mcc* infection with recurrent fever, septicemia, and suspected meningitis in the absence of promoting factors like immunocompromised response or prolonged contact with animals.¹⁸ Isolates have sometimes been reported in unusual animal hosts, such as cattle and wild fauna.^{19–30}

There are still not enough data to accurately estimate the economic consequences of CA in sheep and goats, and the only figure – a mean annual loss of US\$30 million in European countries around the Mediterranean rim – dates from 17 years ago.³¹ A more recent study stated that the cost of one outbreak in mixed sheep/goat farms can range from 7 to 130 k€ depending on herd size and onset of disease in relation to the lactation period.³² CA is already geographically widespread and is poised to become even more important as small ruminant milk production increases worldwide (Table 1).

The prevalence of CA varies hugely between regions across the world, as does the relative importance of each etiological agent. It is important to distinguish sporadic cases, i.e., single outbreaks rapidly and successfully controlled, from endemic areas where effective control measures quickly become very expensive due to too many animals affected or herds mixed during the grazing season in upland farm systems, for instance. Once introduced, CA is always difficult to get rid of. For instance, in the Canary Islands (Spain), CA was first reported in the early 1990s and

is still one of the major small ruminant health problems there today.³⁴ There are only limited data on the relative importance of CA as a health concern in small ruminants as general surveys about mastitis rarely include mycoplasmosis.^{35–37} With respect to clinical or subclinical mastitis, in countries where CA is suspected as endemic, *Ma* is less prevalent than *Staphylococcus* or *Streptococcus* spp. but it remains the second or third etiology to consider, with a more dramatic and longer-term impact on production than other pathogens.^{38–42}

This review aims to update current knowledge of CA and its etiological agents taking into account the most recent developments in research on diagnosis, epidemiology, and control.

Clinical Aspects Etiology

The *Mycoplasma* species involved in CA syndrome depend on the host animal. *Ma* causes disease in both sheep and goats whereas the three other agents are primarily goat pathogens, although they are sporadically evidenced in sheep, especially in mixed breeding environments.^{43–46} Mixed infections involving several *Mycoplasma* species have been reported,^{12–14,47} and the general balance between *Ma* and other members of the *M. mycoides* cluster infection is highly region-dependent.

Clinical Forms

Since the early works, studies monitoring CA clinical features in endemic areas have been scarce.⁴⁸ Prevalence studies are often conducted without taking into account whether there is or not some clinical aspects associated with isolation of mycoplasmas, see for instance,^{46,49–52} while case reports are infrequent and often limited to narrow areas.^{43,53–55} The main trend is that the classical overall picture of clinical CA has changed little, outside a few severe respiratory cases of *Mmc* infection in goats, initially suspected as contagious caprine pleuropneumonia.^{56–59}

In acute CA episodes, mastitis, arthritis, pneumonia, and keratoconjunctivitis are the most frequently reported clinical signs with variations at individual and herd level in terms of presence, association and intensity, depending on whether sheep or goats are affected and on herd size, structure, and husbandry practices.^{48,60} The relative frequency and combination of disorders at different stages of infection are seldom reported but seem to vary strongly,^{61,62} with some outbreaks featuring only one type of sign.^{63,64}

Table I Production Of Milk From Sheep And Goats Worldwide And Its Evolution Between 2005 And 2017 For The First 15 Producers In 2017.

	Milk Production In Tonnes					
	Goats			Sheep		
Year		2017	2005		2017	2005
Total worldwide		18,894,731 (+28%)	14,791,746		11,567,441 (+13%)	10,267,917
Fifteen biggest producers in 2017	India	6,165,500	3,790,000	Turkey	1,344,779	789,877
	Bangladesh	1,113,849	864,389	China	1,166,803	1,114,930
	Sudan	1,109,112	1,519,000	China, mainland	1,166,803	1,114,930
	Pakistan	842,036	675,000	Greece	732,095	752,171
	France	590,000	553,691	Syrian Arab Republic	644,561	765,851
	Greece	562,491	511,373	Romania	527,503	544,400
	Turkey	523,395	253,759	Spain	514,198	407,800
	Spain	491,374	471,900	Iran (Islamic Republic of)	449,718	307,943
	South Sudan	468,000	nk	Italy	410,380	532,049
	Mali	400,487	400,000	Sudan	403,394	487,000
	Somalia	373,828	412,600	Somalia	380,000	475,000
	Niger	362,129	236,004	Mali	364,330	179,470
	Indonesia	361,793	284,459	Algeria	284,684	203,000
	Iran (Islamic Republic of)	311,625	396,242	France	270,000	255,355
	Russian Federation	256,483	253,692	Afghanistan	206,675	167,059

Notes: Countries that are among the 15 biggest producers in 2017 for both sheep and goat milk are in bold characters. Data extracted from Food and Agriculture Organization of the United Nations (FAO). <http://www.fao.org/faostat/en/#data/QL>.³³

Clinical outcome is influenced by but not strictly correlated with the etiological agent involved.⁶⁵ Overall mortality can be high (up to 100%) in young animals^{48,66} but less so in adults, from nil (generally with *Ma*) to 40–50%.^{16,48} Acute and severe forms are considered more frequent with species of the *M. mycoides* group than with *Ma*.^{5,48} Dramatic cases are more frequent in goats whatever the mycoplasma species involved.^{3,5,43,56,58,59}

CA primarily affects lactating females and young animals.¹⁵ Lactating females with CA mainly develop mastitis at an early stage, but the intensity is variable whatever the *Mycoplasma* species involved (*Ma*, *Mmc*, *Mcc*, or *Mp*).^{1,3,48,67} General signs, such as weakness, pyrexia, or anorexia, that precede focal localizations often go unnoticed in adults whereas they can rapidly lead to mortality in young animals.⁴⁸ Arthritis occurs

frequently in young animals as a polyarthritis form causing recumbence, especially with *Mcc*^{1,48} and *Mmc*,^{54,55,64,68} but only occasionally in adults, where it causes lameness and swollen joints affecting mainly the tarsus and carpus.⁶⁹ *Mp* was long thought to cause only mammary disorders until arthritis was also evidenced with *Mp* isolates.^{63,69} Conjunctivitis can occur with *Ma* and less frequently with *Mmc* and *Mcc*^{1,48} but is not often reported as it does not affect herd performance. Pneumonia is less frequent, at least in adults,⁴⁸ but severe pneumonic forms with *Ma*, *Mmc*, and *Mcc* have been described, especially in young goats.^{13,55,69,71} There are sporadic reports of genital,^{12,72} like vaginitis, salpingitis, metritis, testicular degeneration, and neurological disorders.^{73–75}

Clinical signs are frequent during the post-partum period or onset of lactation^{61,76} or against a background of any

immune-depressed conditions such as farm transfer,¹⁵ breakdown in sanitary conditions, or concurrent infection.¹¹

Disease Course

Infection generally onsets with a rapid spread of acute disorders over weeks or months^{11,61} that either recede with time, more or less rapidly depending on the measures implemented, or become recurrent.^{48,77,78} Clinical cases may also appear more gradually or remain sporadic.¹⁵ Course towards chronicity, with attenuated or sporadic disorders, is considered a natural evolution of the disease.^{15,48} It has been reproduced in experimental *Ma* infections where an increase in immune response fails to eliminate the mycoplasmas due to their capacity for immune-evasion.^{79,80} There are also extensive reports of asymptomatic mycoplasmal circulation in goat herds, evidenced mainly in bulk tank milk or ear canal samples, that can persist for years without manifesting any clinical sign, see for instance, Refs. 47,81–85. It can result from past history of the disease in the herd and lead to new clinical CA outbreaks, as carriage strains are as pathogenic as those isolated from acute CA cases.^{82,86}

Transmission

The long persistence of mycoplasmas in clinically CA-infected organs (udders, eyes, respiratory tract) and other biological niches (ear canals) makes the main routes for direct within-herd transmission oral (e.g., colostrum feeding), respiratory (e.g., nasal discharge), and mammary (e.g., kid feeding).^{53,54} The environment, without being a sustainable reservoir, may also play an indirect role in transmission through bedding, the milking parlor, shared feeders, and troughs.⁴⁸ Milking practices and devices are thought to play a highly significant role in indirect transmission.^{11,70,80} Disease transmission between farms frequently occurs with the introduction of asymptomatic carriers.^{11,44,54} As evidenced in other mycoplasmas infecting cattle,⁸⁷ reproduction should also be regarded as a route of introduction or transmission, as semen shedding has been largely demonstrated in goats^{88–94} and appears possible in sheep.^{95,96}

Diagnosis

Clinical Suspicion And Differential Diagnosis

Despite its economic impact worldwide, CA is still under-investigated due to under-awareness by animal health stakeholders, varied clinical impacts, and a lack of financial,

technical, and training means. In typical acute forms, different signs of CA syndrome observed within a herd, i.e., mastitis, arthritis, pneumonia, and keratoconjunctivitis, may prompt a mycoplasma diagnosis.^{1,5} If the disease course remains staggered or if mild signs are observed, then a differential diagnosis is necessary. Several other non-CA agents need to be ruled out, such as (i) *Staphylococcus* and *Streptococcus* spp. in mastitis,⁹⁷ (ii) caprine arthritis encephalitis virus in goat joint lesions,⁹⁸ (iii) *Pasteurellaceae*, parainfluenza, Visna-Maëdi, and “peste des petits ruminants” viruses for respiratory signs,⁹⁹ and (iv) other *Mycoplasma* spp. like *M. conjunctivae* in conjunctivitis alone, *M. capricolum* subsp. *capripneumoniae* the causative agent of contagious caprine pleuropneumonia³ and *M. ovipneumoniae* in respiratory diseases in both sheep and goats.^{57,100,101}

It is often a first-line failure to evidence other pathogens that prompts a mycoplasma investigation. The high variability and low specificity of clinical signs mean clinical suspicion and specific diagnosis may be delayed or even never performed. The problem is compounded by the fact that physiological monitoring as a herd health management tool does not apply for CA. Indeed, in active outbreaks, somatic cell counts (SCC) may increase in correlation with mammary signs^{80,102,103} but remain in normal ranges in chronically infected herds.^{47,85,104,105} Other milk quality traits are affected similarly by mastitis, whatever the etiological agent.¹⁰⁶

Consequently, confirmatory CA diagnosis still relies on conventional laboratory analyses, some of which require specific methods and expertise to rule out other commensal or opportunistic mycoplasmas commonly found in biological samples (e.g., *M. arginini*).

Relevant Samples For Direct Diagnosis

Milk (individual/pooled/tank), joint fluids, and eye swabs are relevant samples in diseased herds, and it is recommended practice to sample several animals in the same herd, due to inter-individual variability in shedding.^{1,107} Excretion in milk is higher in the clinical phase and might become intermittent with time,^{49,83,85,108} necessitating repeated samplings. Eye swabs and joint fluids tend to contain less mycoplasma (especially if lesions are not recent) and do not make the best samples in extensive surveys as they require animal containment or post-mortem sampling.^{43,51,84} Lungs, lymph nodes, and mammary glands can also be sampled post-mortem.¹ Ear canal swabs are not recommended for CA diagnosis as ear canals are a frequent habitat for mycoplasmas in both

healthy and diseased goat herds, and they are difficult to sample reproducibly.^{82,109–111}

Diagnostic Methods

Mycoplasma species involved in CA are relatively easy to cultivate, through liquid-medium enrichment (2–3 days) followed by streaking on agar plates and another 2–3 days incubation to isolate colonies harboring a distinctive morphology.¹ Several media have been developed that contain selective components to inhibit potential contamination of mycoplasma cultures by the sample flora.¹⁷ Identification uses serological (dot immunobinding on membrane filtration, MF-dot or immunofluorescence assay, IFA) or molecular (PCR) methods or MALDI-TOF, as older biochemical testing methods have been abandoned due to over variability.^{17,112} Several CA-agent-specific PCR assays exist and have already been extensively reviewed.¹⁵ However, to date, there is still no one-step technique that can individually identify the four subspecies. A multiplex real-time-PCR method targeting all CA agents (*M. agalactiae* on one hand and the *M. mycoides* cluster on the other hand) has recently been developed and brought to market but it may prove expensive, especially for large-scale screening studies.^{85,113} Performances of all-round PCR diagnosis remain highly dependent on the DNA extraction methods used.^{85,108} With bulk tank milk samples, a combination of culturing and PCR may increase the sensitivity of detection.^{49,108} The high mutation rate in mycoplasma genomes makes it prudent to regularly re-validate available PCRs on newly circulating strains.

Another molecular detection method called Loop-mediated Isothermal Amplification (LAMP) requires less time and equipment than PCR and is used for several human and veterinary pathogens.¹¹⁴ It is portable and usable as a pen-side test for *Ma* detection.^{115,116} Although it holds promise with detection in under 60 mins, it requires further validation on field samples, with special emphasis on the high risk of cross-contamination and subjective reading.¹¹⁴

Indirect methods for diagnosis are also usable in the absence of vaccination at the herd level.^{17,117,118} Commercial ELISAs are currently limited to *Ma* serodiagnosis. They have proven to be more sensitive than complement fixation tests but their performances still require regular testing and validation to adapt to different epidemiological contexts.^{117,119,120} Some are not suitable for early infection detection due to the delayed onset of a serological response.^{120,121} Immunoblotting tests can be used as confirmatory methods but require cumbersome reagent preparation and expertise for reading.^{17,120,122} The recent development of a lateral flow assay as a serological pen-

side test warrants further validation and, to our knowledge, is not yet available as a kit.¹²³

Epidemiology Worldwide (Including Surveillance)

CA counts as one of the 117 notifiable animal diseases, infections, and infestations listed by the World Organization for Animal Health (OIE) in 2019 and has done for years. The Terrestrial Animal Health Code consequently (i) issues recommendations on sheep and goat imports, that include, for instance, the absence of clinical signs on the day of shipment, 6 months of husbandry in a CA-free establishment and in a quarantine station for the 21 days prior to shipment, (ii) repeatedly reviews its protocols for diagnosis and control, and (iii) appoints reference laboratories, which are currently the Istituto Zooprofilattico Sperimentale della Sicilia in Italy alongside the Animal and Plant Health Agency, in the UK. However, as CA is not shortlisted among the six diseases that hold official recognition of status by the OIE, as mandated by the World Trade Organization, no official map of CA prevalence worldwide is published. CA is believed to occur wherever pastoralism and small ruminant dairy production are common, but it is almost certainly underdiagnosed and under-reported. Indeed, if we examine data from small ruminant milk production worldwide, reports of the disease should have increased with the increased tonnage and the presence of several newcomers to the market (Table 1). However, officially (OIE reports), CA has never been reported in India and Bangladesh, is absent from Sudan, and there is no information for Turkey and China, even though these countries make up the big five producers of goat and sheep milk. CA has been regularly reported in Europe, particularly in Mediterranean regions, as well as the Middle East, Asia, North Africa, and South America, through OIE notifications or scientific reports.^{17,62,124,125} It is more sporadic in other countries, like the USA with recent reports¹²⁴ or New Zealand, with no evidence for CA in the last decade. Note that wild fauna may spread the disease or at least act as a reservoir for the etiological agents, but is not specifically surveyed.^{29,126}

Historically and regularly since the 1980s, CA has been described in Mediterranean countries.¹ Figure 1 proposes a snapshot on CA distribution in these countries according to OIE reporting. In the past five years (2014–2018), the disease has been annually reported to the OIE in Greece, Italy, Spain, France, Portugal, Cyprus, Albania, Israel, North Macedonia (until 2015), the Palestinian territories, and Jordan (suspected). For Italy, Greece, and Spain, there are

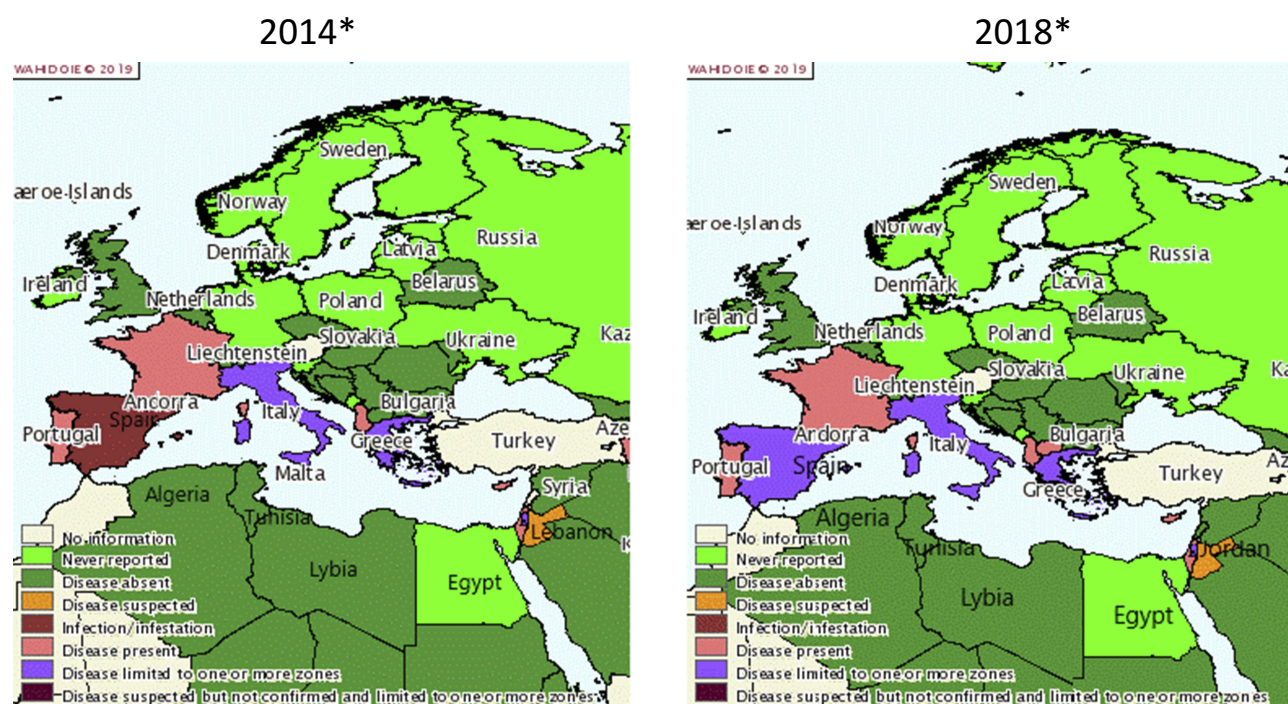


Figure 1 Distribution of contagious agalactia in 2014 and 2018 in Mediterranean countries according to OIE reporting.

Notes: Reproduced with the kind authorisation of the World Organisation for Animal Health [OIE] www.oie.int.¹²⁹ World Organisation for Animal Health (OIE).

partial quantitative data available, and some regions appear particularly affected as they report more often (Sardinia, Andalusia and Castile and Leon, Central Greece and Central, Eastern and Western Macedonia), but the OIE data are insufficient to calculate accurate prevalence. Other countries from Southeast Europe (Bulgaria, Croatia, Slovenia, Montenegro, Bosnia and Herzegovina, and Serbia) and the Maghreb (Tunisia, Algeria, Libya) did not report any CA and some Eastern Mediterranean countries (Lebanon, Egypt, Turkey) either gave no information or no reports. However, the scientific literature does feature clinical cases or surveys of CA for several of these countries: (i) Bosnia and Herzegovina where a survey on respiratory and ocular samples evidenced a few cases of *Mmc*, *Mcc*, and *Mp* in both sheep and goats,⁴⁶ (ii) North Macedonia, where several *Ma* CA cases in sheep and goats were reported,¹²⁷ and (iii) Turkey, where *Ma* appeared to be dominant in both sheep and goats (15% *Ma*-positive in 234 individual samples).^{45,128} This clearly underlines how, despite mandatory notification, CA remains overlooked by national veterinary services.

Furthermore, the official OIE figures do not distinguish the different etiological agents, which have to be investigated through scientific publications. In Spain, the situation depends on the region. In north and central Spanish

provinces, *Ma* is widely present in sheep (37% PCR positive bulk tank milk), but *Mmc*, *Mcc*, and *Mp* remain undetected.⁸³ In Murcia, a goat survey evidenced a high prevalence of CA (67% of the tested farms) dominated by *Ma* (75%) compared to *Mmc* (4%).⁴⁹ In contrast, in the Canary Islands, the prevalence of *Mmc* in goats reached or exceeded *Ma* figures¹¹⁷ as it was documented locally in Gran Canaria where *Mmc* was present in 90% of the CA-positive flocks accounting for 38% of the tested herds.³⁴ In Jordan, a small ruminant survey found mostly *M. mycoides* cluster species and few *Ma* cases.⁴³ In France, the “Vigimyc” surveillance network monitors the CA situation at a national level, except for the Pyrénées-Atlantiques region.^{44,57} Vigimyc is a voluntary-basis network for collecting and identifying isolates from clinical cases, so it cannot serve to assess prevalence but it can give an idea of the relative proportions of each species in CA cases: *Mmc* (42%), *Mcc* (26%), and *Mp* (15%).⁴⁴ Unlike in Spain, *Ma* is rarely isolated in goats with clinical signs. These trends have remained relatively stable since 2010 (unpublished data). Most ovine clinical cases covered by the network concern respiratory disorders. In the Pyrénées-Atlantiques, which is a major French sheep-breeding region, CA is endemic in sheep but the prevalence of *Ma* infection has

fallen below 5% after several years of control measures (see <http://www.gds64.fr/maladies-actions-sanitaires/ovins-caprins/agalactie-contagieuse/les-actions/>). In other countries, in the absence of surveys simultaneously targeting all CA mycoplasma species, their current relative distributions remain unknown. In Italy, for instance, studies have focused mostly on *Ma* where it is considered as dominant, in Sicily and Sardinia.¹³⁰ *Ma* strains have been indeed isolated from sheep in several regions including Sardinia, Lazio, Sicily, and Puglia,^{103,131,132} whereas *Mmc* strains were also isolated in goats in Sardinia and Sicily.^{54,133,134} In 2016, a random *Ma* survey (PCR on bulk tank milk) conducted in Sardinia in both sheep and goats found relatively low prevalence rates in sheep 4.8% (n=1064 farms) and goats 4.5% (n=66 farms).¹³⁵ Likewise, in Greece and Cyprus, all the available studies in both sheep and goats have focused exclusively on *Ma*.^{40,53,136,137} Consequently, the statement that *Ma* is the dominant CA etiology in the Mediterranean area¹³⁰ should be revised, as while it may be true for sheep (in which *Ma* is the only etiological agent), it is not the case in goats. Potential differences in breed susceptibilities versus one or the other etiological agent have yet to be explored although they are poorly probable due to previously described clinical cases in herds with mixed breed.^{15,54}

Recent Advances In The Biology Of The Etiological Agents

The etiological agents of CA all belong to the *Mycoplasma* genus, a group of bacteria that has evolved from a Gram-positive ancestor, through successive and severe gene reduction, resulting in their current form, which are bacteria with small genomes (ca. 0.5–1.5 Mbp),¹³⁸ no cell wall,¹³⁹ and reduced metabolic pathways.¹⁴⁰ These characteristics have forged a parasitic life-style with huge dependence on the animal-host for nutrients.

Taxonomy

The taxonomic status of the *Ma* species has remained unchanged since it was first established in the *Mycoplasma* genus in the 1950s. There used to be a subspecies, named *M. agalactiae* subsp. *bovis* that rapidly became the *M. bovis* species, which is phylogenetically very close to *Ma* and involved in bovine pneumonia, arthritis, and mastitis.¹⁴¹ In contrast, the taxonomy and phylogeny of the other etiological agents have been revised extensively over the years with for instance *M. mycoides* subsp. *mycoides* Large

Colony biotype (*Mmm*LC) and *M. mycoides* subsp. *capri* (*Mmc*) being grouped under one unique subspecies, *Mmc*.⁸ *Mmc*, like *Mcc*, is part of the *M. mycoides* cluster, a group of closely related species that also includes the etiological agents of contagious bovine and caprine pleuropneumonia and a potential fourth member, *M. feriruminatoris* subsp. *nov*.^{7,8,142} However, *M. putrefaciens* the fourth etiological agent of CA does not strictly belong to the cluster, and its relative positioning has been debated several times.^{7,142}

Genomic Plasticity

The theory of a reductive evolution of mycoplasmas with genetic erosion leading to extinction, clonality, or intracellularity has been challenged in recent decades, and, today, at least one genome has been sequenced for each species (Table 2). Contrary to other bacteria genomes, plasmids are poor contributors to genome diversity and dissemination of beneficial traits, such as antimicrobial resistance, as they are found only in the *M. mycoides* cluster and have a small size with no cargo gene.¹⁴³ In silico comparative genomic analysis has pointed out massive horizontal gene transfers between different species sharing the same host.^{144,145} Around 130 genes in *M. agalactiae*, for example, are thought to originate from the phylogenetically remote *M. mycoides* cluster. This gene transfer was later confirmed in vitro, using *M. agalactiae* as a model organism, and associated with the presence of self-transmissible mobile genetic elements called Integrative Conjugative Elements (ICEs).^{146,147} ICEs encode their own excision, transfer by conjugation, and integration into the recipient-cell chromosome. Once in contact, the recipient and donor cells can also exchange through an unconventional conjugative mechanism of chromosomal transfers (CTs) which involves large chromosomal regions, whatever their genomic locations.¹⁴⁷ These CTs, which can comprise up to 10% of the genome, generate highly mosaic genomes, leading to a concept from Citti et al that

mycoplasma populations can be seen as a dynamic gene pool compensating genome erosion as well as clonality, allowing horizontal dissemination of genetic traits that may in turn result in the emergence of new strains, with new properties.^{148,149}

All this has been demonstrated in vitro and mainly for *M. agalactiae*, but the same process can reasonably be hypothesized in other mycoplasma species, as ICEs are relatively prevalent in ruminant mycoplasmas.¹⁵¹ Conjugative transfers of ICEs or CTs between *M. mycoides* and *Ma* have

Table 2 List Of Strains With A Sequenced Genome, Among The Etiological Agents Of Contagious Agalactia, With Main Characteristics Of The Genomes. All Strains Were Originally Isolated From Goats

(Sub)Species	Strain			Genome				
	Name	Isolation Place, Date	Clinical Signs	RefSeq Accession And Ref	Size (bp)	GC Content	Total Number Of CDS	Mobile Genetic Elements
<i>M. agalactiae</i>	PG2	Spain, 1952	nk, type strain	NC_009497 ¹⁴⁴	877,438	29.7	752	No plasmid, one vestigial ICE
<i>M. mycoides</i> subsp. <i>capri</i>	95010	France, 1995	Arthritis	NC_801543 ²²¹	1,153,998	23.8	924	One plasmid of 1,840 bp (pMmc-95010), ICEM, 2 copies
<i>M. capricolum</i> subsp. <i>capricolum</i>	California Kid	USA, 1955	Arthritis, type strain	NC_007633 ²²²	1,010,023	23.8	827	No plasmid, ICEC, 1 copy
<i>M. putrefaciens</i>	KS1	USA, nk	nk, type strain	NC_015946 ¹⁵⁰	832,603	26.9	650	No plasmid, nor ICE

Abbreviations: ICE, integrative conjugative elements; nk, not known.

never yet been demonstrated in vitro or in vivo. Further investigation is warranted and would be key to understanding the evolution of strains within the goat host, which might be favorable to horizontal gene transfers between *M. mycoides* and *M. agalactiae*, in contrast to the sheep host where *Ma* the sole CA agent.¹⁵² Indeed, clonal spread of *Ma* in sheep has been reported several times at different scales, from an endemic area up to country-wide level,^{29,153–155} whereas caprine isolates are described as more variable.^{152,156} The development of new molecular genotyping methods such as multiple locus variable number of tandem repeats analysis (MLVA) or multilocus sequence typing (MLST) with a better discriminatory power than restriction endonuclease-based techniques, size variation in *vpma* genes repertoire, Insertion Sequence (IS) typing or random amplified polymorphic DNA patterns has brought clearer insight into *M. agalactiae* diversity.^{154,157,158} In contrast, there is still no approved MLST or MLVA scheme for members of the *M. mycoides* cluster despite one proposal for an MLST scheme.^{7,159} With the decrease of whole-genome sequencing costs, core genome MLST, as developed for the poultry pathogen *M. synoviae*, should become a more accurate alternative to MLST.¹⁶⁰

Another feature of mycoplasma genomes is their fast evolution, with one of the highest bacterial mutation rates due to their degenerated DNA repair systems. *M. gallisepticum*, for example, has a nucleotide substitution rate of $0.5\text{--}1.2 \times 10^{-5}$ per site per year whereas *Staphylococcus aureus* has a rate of $1.2\text{--}2.6 \times 10^{-6}$.¹⁶¹ This has implications for PCR-based diagnosis since mismatches can occur in the primer hybridization

sequence and thus erode the specificity of the PCR assays, as illustrated by several studies.^{9,162}

Virulence

The pathological effects of mycoplasma infections are deemed to result more from an inappropriate response of the animal host following colonization rather than production of mycoplasmal virulence factors with cell-toxic effects.¹⁶³ Efficient colonization and persistence in the host are key steps in mycoplasmosis, and they are mainly mediated by efficient nutrient scavenging, adhesion to the host cells, and immune evasion. Intracellular invasion has been documented in various species from the avian pathogen *M. gallisepticum*¹⁶⁴ to more recently the porcine agent *M. hyopneumoniae*,¹⁶⁵ but its role in pathogenesis is currently unknown. In *Ma*, the immune evasion is largely mediated by variable surface proteins, called “Vpma”, and phase variations of capsular coating.^{166,167} The Vpma expression profiles at different stages and different sites of ewes experimentally infected with *Ma* changed very rapidly, preventing efficient immune response.¹⁶⁸ Furthermore, Vpma were shown to play a role in cell adhesion and invasion; hence, their variation is important in pathogenesis.¹⁶⁹ In *Ma*, adhesion is also mediated by other well-described adhesins, like P40.¹⁷⁰ Another original but complex system of immune evasion has recently been evidenced in mycoplasmas, the so-called MIB–MIP system able to cleave off the VH domain of the host immunoglobulin G.¹⁷¹

For years, the lack of genetic tools to engineer mycoplasma genomes has delayed formal characterization of virulence traits *in vivo*. However, recent advances in synthetic biology and in editing of mycoplasma genomes cloned in yeasts have opened new possibilities.^{172,173} Very recently, Jores et al succeeded in excising 68 non-essential genes accounting for ca. 100 kbp from the JCVI-Syn3.0 mutant of *Mmc* GM12 and generate a fully attenuated strain.¹⁷⁴ Cumulative deletion of 5 candidate virulence traits, like the glycerol-dependent hydrogen metabolic pathway, 9 lipoproteins including major antigens (LppA/P72), mycoplasma-specific F1-like X0 ATPase, the MIB-MIP system, an ICE, and other Lpps including lppQ failed to specifically designate which deletion is responsible for attenuation in a challenge goat model.

All this host-mycoplasma interplay has a huge impact on clinical expression of the disease, resulting in long-term persistence within a host or a herd, intermittent excretion and an immune response that fluctuates over time. All these parameters also influence the efficiency of diagnostic tools.

Means And Relative Effectiveness Of Control

Control of CA relies on culling the affected flocks, chemotherapy and/or vaccination, or combinations of all three measures, but there is practically no international coordination on the issue, with each country opting for its own way depending on pattern of infective spread (enzootic area versus sporadic cases). Good herd hygiene management practices and biosecurity measures are also key, as once introduced in a herd, the disease may spread fast.^{15,54} Eradication of CA requires sustained financial resources and long-term commitment from the stakeholders, which creates another additional cost that is rarely taken into account.^{15,32,130}

Vaccines

For a number of years, relatively ineffective mycoplasma vaccines were produced directly from milk or other tissues of infected small ruminants.¹⁷⁵ They were then banned as they were suspected to be responsible for increase in scrapie outbreaks in Italy. From then on, several assays were performed to prepare safe and effective vaccines, mainly against *Ma* CA.^{176–183} Live attenuated vaccines, such as the ones used in Turkey, were reported to be more efficient in the long term than inactivated vaccines.^{31,184} However, they are not authorized in many countries and should not be used during the lactation period. Hence,

most developments have focused on inactivated vaccines either for local use (autogenous vaccine) or for licensing. However, even today there is still no effort to standardize an assessment method, nor to harmonize regulatory constraints worldwide, and still there is not a single universally recognized vaccine.¹⁷

The strain included in the vaccine as well as the inactivation method and the adjuvant used are all key factors for immunogenic efficacy against potentially very variable infective strains.^{184–186} Mineral-oil adjuvant-inactivated vaccines induce higher and longer-lasting protective immunity than the aluminium-hydroxide-absorbed vaccines, but they can also induce lesions at the injection site.^{176,179,182,187} Inactivated vaccines remain sub-optimal, as most of the time they reduce clinical severity rather than preventing new infections or even milk excretion.^{49,180,184} The quick decrease in antibody titers, frequently followed by a generic ELISA test that gives no indication on their protective nature, imposes repeated vaccinations every 6 months. Consequently, vaccination should be combined with chemotherapy to improve the chance of both clinical and microbiological cure.

There has been little attempt to develop vaccines against the *M. mycoides* cluster species except one in India against *Mmc*.¹⁸⁸ Licensed vaccines can be polyvalent with proven efficacy in an endemic area¹⁸⁰ or monovalent but actually claiming to cross-protect against different *Mycoplasma* spp.¹⁸⁹ The development of synthetic biology to customize strains might be a promising approach for tomorrow's vaccines.¹⁷⁴

Autogenous vaccines against CA have attracted little attention in the literature, and case studies or experimental challenge reports are scarce.^{184,190} As a rule, autogenous vaccines are considered a useful addition or feasible substitutes to licensed vaccines,¹⁹¹ but only bring local solutions, in farms that have already suffered an outbreak. They are especially considered in *M. mycoides* CA cases as there is no commercial vaccine.

Antimicrobials And Resistance

Tetracyclines, macrolides, and fluoroquinolones are currently the top recommended drugs against CA,^{14,15} while other molecules, such as florfenicol and tiamulin, were more popular in the past.⁴⁸ Papers have long underlined the importance of early, collective and repeat administrations for therapeutic success at the herd scale.¹ Unfortunately, actual use of antimicrobials and the clinical and microbiological outcomes are rarely reported. There

are few antimicrobials available with a market authorization specific for small ruminants. Most of these shortfalls are tackled using products authorized in cattle, rationalized on the cascade principle. Antimicrobials do allow clinical recovery but rarely complete bacteriological clearance,⁴⁸ as nicely illustrated by two recent reports of bitherapy with tetracycline and macrolides.^{54,69} A study on *M. bovis*, another ruminant mycoplasma very closely related to *Ma*, showed that the level of resistance for almost all antimicrobials except fluoroquinolones has increased dramatically in contemporary isolates.¹⁹² In this context, antimicrobial susceptibility testing (AST) of CA agents is vital, but has only been tackled in a few studies.^{152,193–200}

High-throughput AST techniques such as the disk diffusion method cannot be used for *Mycoplasma* spp. due to their slow growth and the need for complex medium. AST thus hinges on minimum inhibitory concentration (MIC) testing, either by agar or micro-broth dilution, for which specific recommendations have been issued.²⁰¹ The lack of harmonized procedures and quality controls for veterinary mycoplasma AST rules out good data comparison, and, within veterinary mycoplasmas, species affecting small ruminants are often neglected.²⁰² A further barrier is the absence of clinical breakpoints for interpretation of MICs in terms of therapeutic efficacy in vivo (resistant intermediary, or susceptible). For some species harboring a single tissue tropism, breakpoints for other pathogens with similar biological niches have been used (e.g., *Pasteurellaceae* for *M. bovis*),^{192,203} but this approach is not feasible with CA agents due to the diversity of their body localization.

Table 3 summarizes MIC values from different studies for each etiological agent of CA stratified by the main antimicrobials used in small ruminants. The general picture is a dominant low-MIC population of strains with a secondary, limited (especially for phenicols and fluoroquinolones) population exhibiting moderate MIC increases for most antimicrobial families. There are slight differences between CA species, but overall the shift to high MIC only concerns a few strains, which is a very different picture to *M. bovis* for which most current strains are resistant.²⁰² For oxytetracycline, the most widely used tetracycline, tylosin, the most widely used macrolide, and lincomycin, the most widely used lincosamide, regardless of mycoplasma species, the dominant population has low MICs (≤ 1 $\mu\text{g/mL}$). A shift toward higher values (MIC₉₀ between 2 and 16 $\mu\text{g/mL}$) was evidenced in *Ma*, *Mcc*, and *Mp* except for *Mp* with tylosin, see, for instance, Refs. 152, 159, 204. For oxytetracycline, MIC values were higher for *Ma* than for the *M. mycoides* cluster.^{195,198} Poumarat

Table 3 Overview Of MIC Distribution Of CA Mycoplasma Species (Microbroth Or Agar Dilution Methods Depending On The Study) And Main Molecular Resistance Mechanisms When Known

Mycoplasma Species	Fluoroquinolones		Phenicols		Tetracyclines		Macrolides		Lincosamides		Aminosides	
	ENRO	Mec.	MIC Range MIC ₉₀ Range $\mu\text{g/mL}$	Mec.	OXY	MIC Range MIC ₉₀ Range $\mu\text{g/mL}$	Mec.	TYL	MIC Range MIC ₉₀ Range $\mu\text{g/mL}$	Mec.	SPT	Mec.
<i>Ma</i> 152,196,198,199,204, 195	<0.125–0.8 0.1–0.5	Mutations in QRDR	1–8 2–8	ns	0.06–8 0.5–8	0.025–16 0.25–8	rrs mutations	0.025–16 0.25–8	0.125–4 1–4	rrn and L22 coding gene mutations	0.4–12.8 2–8	ns
<i>Mcc</i> 200,206,207	0.025–3.2 0.25–0.4	Mutations in QRDR	4–8 8	ns	0.25–4 0.5–2	0.05–12.8 ≤ 0.03 –12.8	na	0.05–12.8 ≤ 0.03 –12.8	0.2–12.8 12.8	rrn and L22 coding gene mutations	16–128 128	ns
<i>Mmc</i> 194,198,200, 197	0.025–0.2 0.1–0.125	na	2–4 4	ns	0.125–4 0.25–0.5	0.025–1 0.06–1	na	0.025–1 0.06–1	0.125–12.8 0.5–0.8	rrn and L22 coding gene mutations	8–128 16–64	ns
<i>Mp</i> 193,198,200	0.06–0.25 0.125–0.25	na	0.5–4 1	ns	0.250–8 0.5–4	0.015–2 0.015–0.12	na	0.015–2 0.015–0.12	0.250–16 1–2	rrn and L22 coding gene mutations	8–32 16–32	ns

Abbreviations: Mec., mechanism; ns, not studied so far; na, not applicable; ENRO, enrofloxacin; FFC, florfenicol; OXY, oxytetracycline; TYL, tylosin; LIN, lincomycin; SPT, spectinomycin.

et al also reported higher MICs with oxytetracycline but lower MICs with tylosin for *Ma* in goats versus sheep, which may reflect the different use of the molecules.¹⁵² The bimodal distribution of MICs with higher values ($8 \leq \text{MIC}_{90} \leq 32 \mu\text{g/mL}$) is also observed for other 16-membered ring macrolides like spiramycin in all species except *Mp* and tilmicosin for *Ma* and *Mcc*.^{152,159,196,197,199} For the latest generation macrolides, represented by the long-acting tulathromycin, some increased MICs have been documented with *Ma* but they remained limited ($[2-8] \mu\text{g/mL}$).^{152,196} No increased-MIC population has been observed yet with the less-used second-generation tetracycline, doxycycline, as there is no MIC above $1 \mu\text{g/mL}$, see, for instance, Refs.¹⁹⁸ and ¹⁹⁹ All MICs for aminosides were high for species of the “*M. mycoides*” cluster ($\text{MIC}_{90} > 16 \mu\text{g/mL}$), which is consistent with a suspected intrinsic resistance of other species within this group.²⁰⁵ For *Ma*, MICs with aminosides are broadly distributed, with infrequent reports of increased MICs.^{152,195,196,199} MIC values with phenicols mostly range between 1 and $8 \mu\text{g/mL}$ with a limited MIC increase (MIC_{90} reaching 4 or $8 \mu\text{g/mL}$) whatever the mycoplasma species.^{152,196,200} Finally, for third-generation fluoroquinolones, there is a less marked-shift towards increased MIC values, with MIC remaining low ($\leq 0.5 \mu\text{g/mL}$) except in a few Spanish *Mcc* and *Ma* strains with values between 1 and $4 \mu\text{g/mL}$.^{159,196}

As described in other species,²⁰² CA mycoplasma resistance hinges mostly on point mutations affecting the antimicrobial binding target. Macrolides and lincosamides resistance correlate with mutations in domain V of the 23S rRNA and ribosomal protein L22 in *Ma* and *Mcc* isolates.^{204,206} Increased MIC values with third-generation fluoroquinolones are associated with mutation in the quinolone resistance determining regions of *gyrA* (Glu87Lys), *gyrB* (Pro343Leu), and *parC* (Ser80Thr or Asp84Gly) in *Ma* and *Mcc* field isolates.²⁰⁷ For oxytetracycline, the situation is more complex. In some *Ma* strains, but not all, the classical hot-spot mutations in the Tet-1 domain in one or both *rrs* allele(s) have been associated with resistance.¹⁹⁸ In the *M. mycoides* cluster, strains with increased MICs do not harbor any mutation in or outside Tet-1,¹⁹⁸ suggesting the existence of other resistance mechanisms. Mechanisms for phenicol and aminoside resistances remain unexplored.

Other Compounds With Antimicrobial Effect

Several recent studies have assessed the in vitro antimycoplasmal efficacy of plant extracts against *Mcc* and *Mmc* as an

alternative to antimicrobials²⁰⁸⁻²¹³ including one field trial with promising results.²¹⁴ These studies focused on endemic plants generally used by the local populations for medicinal purposes, which is also a promising approach given how access to veterinary drugs is critical in many small ruminant breeding regions of the world. Studies have evidenced anti-mycoplasmal activities of several plant extracts using recommended AST methods.^{208,209,211} MIC ranged between $3.1-12.5 \text{ mg/mL}$ and $0.05-0.6 \text{ mg/mL}$ for *Mcc*,^{208,211} and $0.001-1 \text{ mg/mL}$ and $0.13-0.6 \text{ mg/mL}$ for *Mmc*.^{209,211} The efficacy of plant extracts appears to be highly dependent on extraction process and storage conditions.²⁰⁹ Given their high inhibitory concentrations compared to conventional drugs, further investigations are needed to gauge their safety for animals and their bioavailability.

Good Herd Management Practices And Animal Welfare

In addition to antimicrobial treatment and vaccines, herd management practices are helpful in confining the disease within an infected herd and preventing the introduction of infected animals.

As demonstrated experimentally with *Ma* and *Mmc*, colostrum heating (56°C , 60 mins) reduces mycoplasma load and thus limits offspring oral infection.²¹⁵ The reduction of mammary transmission between adults requires checking milking equipment and process hygiene (e.g., milking order, parlor, cleaning devices and milkers' hands, discarding foremilk, thorough teat cleaning and dipping).¹¹⁸ Isolating clinically affected animals is generally recommended but is rarely practicable when clinical forms are widespread. Regular disinfection of the farm environment (beddings, feeders, troughs), and especially the milking device, can help limit indirect transmission. Several disinfectants have proven to be efficient in vitro against *Mycoplasma* spp.^{216,217} but their in-field implementation has yet to be validated. A control and slaughter strategy aiming to eliminate infected and/or debilitated animals could be a good complementary measure to recover production capacity or further achieve eradication. The detection of shedders requires repeated individual or bulk direct testing due to the intermittency of excretion, which is also applicable to introduced animals. All introduced animals should be monitored, at least in quarantine.⁵⁰ Rather than individual analyses, pre-movement testing in supplier herds needs to be repeated to reduce the risk of introduction.⁸⁵ As semen shedding maybe an issue (see paragraph on transmission routes), these measures

should also be applied to males temporarily lent out for mating or to semen suppliers.^{90,91}

Stress-limiting breeding practices could promote clinical recovery (quality of feeding and health management, environmental conditions). A recent assessment of small ruminant welfare in current breeding systems challenges some of the commonly recommended herd management practices and control measures as they run counter to natural behaviors.^{218,219} For instance, goats are naturally more reactive, aggressive and exploratory than sheep, and hence their space provided per animal is critical. Goats need to maintain vocal, visual and olfactory contacts with other individuals, so any isolation (kids from their mothers or bucks from does) or regrouping practices will be stressful. Intensive indoor housing systems provide control of feeding regimes, climate, and parasite loads, but they also put constraints on naturalness. A good compromise between animal needs and natural social organization and management systems can reduce stress experienced by the animals, which could in turn limit their tendency to get sick, especially from mycoplasmosis that often results from stress-driven non-adapted immune response, as observed in small ruminants¹³ as well as in cattle.²²⁰

Conclusions And Knowledge Gaps

Small ruminant livestock is distributed worldwide, including in many low-income countries, some of which are currently switching from traditional, extensive, familial systems to semi-intensive or intensive farming. This trajectory raises the likelihood of increasing CA risk with animal grouping. Better follow-up of the true prevalence of the disease worldwide and its associated economic burden is needed to support this transition, and should ultimately help raise awareness on CA of the regulatory authorities.

CA has been known for a long time, yet it is still a neglected disease worldwide. It is difficult to prevent and control due to its rapid spread, multiple sources of infection with both horizontal and vertical transfer, multiple potential etiological agents with different antimicrobial susceptibilities, and different forms of the disease from acute to sub-acute, and chronic or asymptomatic.

Diagnostic tests are underperformed due to the cost and expertise required, creating a need to develop affordable new pen-side tests. Although the etiological agents remain susceptible to most antimicrobial families, surveillance of resistance is important, as some resistant clinical isolates have emerged that will require efforts to harmonize the techniques and develop easy-to-perform tests. There is still no universal vaccine available, despite huge efforts to develop one, at least

against *Ma*. Hence, in 2019, there is still a pressing need for validated low-cost vaccines and a proper vaccination scheme suitable for low and middle-income countries. With recent progress in genome manipulation, tailoring genetically attenuated strains should be reconsidered as an option.

Concerning the agents *per se*, there are still a number of unresolved questions concerning circulation in the host, interplay with the immune system, shedding, and infectious dose for various transmission routes. The development of genome editing tools might help to decipher the virulence determinant of different strains, and all these data are expected to have a tangible impact on CA prevention and control.

Last but not least, CA poses a set of challenges in terms of growing consumer concern for the livestock welfare. The disease affects all three pillars of welfare: the animal's physical state (whatever its clinical form), affective state (with chronic debilitation), and behavioral needs which are compromised by the herd management practices imposed by CA. So far, there has been little if any effort to frame CA as an animal welfare issue.

In conclusion, the old CA disease that emerged in the early 19th century will continue to require collective commitment from breeders, veterinarians, scientists, and regulatory authorities through the next coming decades.

Disclosure

The authors report no conflicts of interest in this work.

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