Population structure and virulence gene profiles of Streptococcus agalactiae collected worldwide from different hosts

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Abstract: Streptococcus (S.) agalactiae is a leading cause of morbidity and mortality among neonates and causes severe infections in pregnant women and nonpregnant predisposed adults, as well as various animal species worldwide. Still, information on the population structure of S. agalactiae and the geographical distribution of different clones is limited. Further data is needed to identify particularly successful clones and obtain insights into possible routes of transmission within one host species and across species borders. We aimed to determine the population structure and virulence gene profiles of S. agalactiae strains from different sources and geographical origins. To this end, 373 S. agalactiae isolates from humans and animals from five different continents were typed by DNA microarray profiling. A total of 242 different strains were identified. Particularly successful clonal lineages, hybridization patterns, and strains were identified that were spread across different continents and/or were present in more than one host species. The findings of our study suggest that while S. agalactiae is well adapted to various hosts (including humans, cattle, dogs and other species), interspecies transmission is possible and occurs between humans and cows, dogs, and rabbits. The presented virulence and resistance gene profiles enable new insights into interspecies transmission and make a crucial contribution in the identification of suitable targets for therapeutic agents and vaccines.

Population structure and virulence gene profiles of *Streptococcus agalactiae* collected worldwide from different hosts

Running Title*:* DNA microarray typing of GBS

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Abstract

Streptococcus (S.) agalactiae is a leading cause of morbidity and mortality among neonates and causes severe infections in pregnant women and nonpregnant predisposed adults, as well as various animal species worldwide. Still, information on the population structure of *S. agalactiae* and the geographical distribution of different clones is limited. Further data is urgently needed to identify particularly successful clones and obtain insights into possible routes of transmission within one host species and across species borders. We aimed to determine the population structure and virulence gene profiles of *S. agalactiae* strains from a diverse set of sources and geographical origins. To this end, 373 *S. agalactiae* isolates obtained from humans and animals from five different continents were typed by DNA microarray profiling. A total of 242 different *S. agalactiae* strains were identified and further analyzed. Particularly successful clonal lineages, hybridization patterns, and strains were identified that were spread across different continents and/or were present in more than one host species. In particular, several strains were detected both in humans and cattle, and several canine strains were also detected in samples from human, bovine, and porcine hosts. The findings of our study suggest that while *S. agalactiae* is well adapted to various hosts including humans, cattle, dogs, rodents, and fish, interspecies transmission is possible and occurs between humans and cows, dogs, and rabbits. The presented virulence and resistance gene profiles enable new insights into interspecies transmission and make a crucial contribution in the identification of suitable targets for therapeutic agents and vaccines.

Keywords: genotype B *Streptococci*, GBS, transmission, capsular serotype, resistance, clonality

Introduction

Streptococcus (S.) agalactiae, also known as group B *Streptococcus* (GBS), emerged in the 1970s as a major cause of morbidity and mortality in neonates and pregnant women. The organism leads to meningitis and septicemia in newborns and severe peripartum complications in pregnant women [1]. *S. agalactiae* has been linked to disease in the elderly and in nonpregnant adults suffering from chronic diseases [2,3]. The organism is also commonly found in food [4] and there are some indications for foodborne/ feedborne transmission [5–7]. In spite of numerous eradication programs, *S. agalactiae* is still a common cause of bovine intramammary infections in many countries [8], with particularly high herd prevalence levels in countries with emerging dairy industries [9].

Capsular polysaccharide (CPS) was recognized as a major virulence factor of *S. agalactiae* and plays an important role in the evasion of host defence mechanisms. CPS has also been used to type GBS and assign isolates to distinct CPS serotypes (Ia, Ib, and II to IX), with serotypes Ia, Ib, II, III and V being highly prevalent in human invasive GBS isolates in many regions of the world [10–12]. Vaccines combining these serotypes can be highly effective, they fail however to offer protection against other GBS serotypes, which cause the majority of GBS infections in some regions of the world such as Japan [11,12].

GBS strains can harbour a wide range of genes encoding virulence factors such as Bac involved in immune evasion, the alpha-like proteins involved in invasion, or the pilus islands, which play a role in host adaptation and specificity. GBS also frequently exhibit resistance genes, including genes conferring resistance to macrolide, lincosamide, and tetracycline. Recently, several studies typing and characterizing *S. agalactiae* isolates have been published [13–19] and a tool for rapid GBS typing based on DNA microarray hybridization patterns (HPs) has been introduced [13]. However, comprehensive information on the population structure and virulence gene profiles of *S. agalactiae* and the geographical distribution of

different clonal lineages is extremely scarce. In particular, comprehensive data on the population structure and virulence gene profile of isolates from a broad range of host species is missing. This data would be crucial to obtain further insights into host adaptation, to identify particularly successful clones, and to determine the geographical distribution of different clonal lineages. It could also be used to identify suitable targets for vaccines and antimicrobial agents, and to further elucidate possible routes of transmission.

A prospective cross-sectional cohort study found that exposure to cattle is a predictor of human colonization with *S. agalactiae* [20]. Case reports and some GBS typing data indicate possible transmission not only between human hosts and cows, but also human hosts and dogs, cats, and crocodiles [21–25]. In addition, experimental studies have evidenced transmission of bovine and human *S. agalactiae* strains to fish [26–28]. Still, data on interspecies transmission is scarce and strain typing studies involving a diverse set of hosts and geographical areas are missing.

Therefore, here we provide data on the population structure and virulence gene profiles of *S. agalactiae* strains isolated from a diverse set of hosts and a wide variety of geographical areas.

Material and methods

Bacterial isolates

In this study, a total of 373 *S. agalactiae* isolates from 5 different continents were analyzed. Countries of origin represented in this study were: Belgium (n = 1), Colombia (n = 86), Costa Rica (n = 1), Germany (n = 109), Honduras (n = 3), Hong Kong SAR, China (n = 30), Kenya (n = 33), Switzerland (n = 103), Thailand (n = 6), Vietnam (n = 1). Isolates included in this study originated from human hosts (n = 225), cattle (n = 84), dogs (n = 16), fish (n = 15), mice (n = 11), elephants (n = 7), guinea pigs (n = 3), emerald monitors (n = 3), rats (n = 2), snakes (n = 2) and one isolate each was collected from a rabbit, a goat, a pig, a turtle, and a frog. A full summary stating the host species, geographical source, and sample type is provided as Online Resource 1.

DNA extraction and DNA microarray

All isolates other than fish isolates were cultivated on 5% sheep blood agar (Oxoid Limited, Hampshire, UK) and incubated for 48 to 72 hours at 37°C. *S. agalactiae* isolates obtained from fish were streaked on both sheep blood agar and Tryptic Soy Agar (Becton Dickinson), and incubated for 72 hours at 30°C. Subsequent DNA extraction was performed using a Qiagen DNeasy kit and following the recommendations of the DNA microarray S.agaType AS-1 kit provider (Alere Technologies, Jena, Germany). As this protocol proved unsuccessful in fish isolates, these isolates were cultivated in 10 mL Tryptic Soy Broth and/or 10 mL Brain Heart Infusion and incubated at 28°C and at 200 rpm/min for 48h or until clouding of the broth culture was visible. The following day, cells were harvested by centrifugation and dissolved in A1 lysis buffer, before transfer to the A2 lysis enhancer Eppendorf tube, to which 20 µL achromopeptidase was added. Subsequent steps were performed according to the manufacturer`s protocol (Alere Technologies). A ND-100 UV-Vis

141 spectrophotometer (NanoDrop Technologies, Wilmington, Germany) was used to measure
142 DNA concentrations in all samples.
143 The DNA microarray used in this study provides data on the presence/absence of typing
144 markers (capsule/pilus-associated genes and *alp* genes), as well as genes conferring resistance
145 (resistance to macrolide/ lincosamide antibiotics, tetracycline, heavy metals) or encoding
146 virulence factors, enzymes and other metabolic functions [13]. Linear PCR amplification and
147 DNA microarray hybridization, washing steps, and staining were performed as suggested by
148 the DNA microarray manufacturer. Hybridization patterns and signal intensities were
149 measured applying an ArrayMate reader (Alere Technologies) and were used for *S. agalactiae*
150 species confirmation, assignment to a clonal complex and capsule type, hybridization pattern,
151 and strain, where possible [13].
152

153 **SplitsTree analysis**

154 Similar to Coombs et al., DNA microarray hybridization profiles were used to
155 calculate unrooted phylogenetic networks from molecular sequence data [29,30]. Stringent
156 inclusion criteria were applied to avoid bias. Multiple isolates were considered to represent
157 the same strain (*e.g.* S1) if DNA microarray hybridization results were identical for all
158 positive/negative signals. In these cases, only one *S. agalactiae* DNA microarray profile was
159 considered for construction of the SplitsTree and was included in the statistical analysis. This
160 resulted in a total number of 161 strains from humans, 52 strains from ruminants, 15 strains
161 from dogs, 8 strains form rodents, 8 strains from fish, and 12 strains from other hosts being
162 included in the statistical analysis. SplitsTree4 (www.splitstree.org) was used to depict the
163 degree of similarity of the different *S. agalactiae* hybridization patterns [31].
164

165 **Statistical analysis**

Statistically significant differences ($p \leq 0.050$) in the distribution of virulence and resistance genes between isolates from different sources (hosts or host groups) were determined either by Chi squared test or Fisher's exact test (in case $n < 5$) using SPSS 24.0 (IBM Corp., Armonk, NY, USA).

Results

The 373 GBS isolates included in this study could be assigned to 242 different strains. Multiple isolates representing the same strain were detected in many host species and across different countries or continents (see Table 1). We observed particularly high rates of duplicates assigned to the same strain among murine (64%), piscine (47%), and bovine isolates (39%). In addition, isolates representing the same *S. agalactiae* strains were not only detected multiple times within one host species, but in some cases also across different host species (see Fig. 1).

We determined pronounced host-specific differences in the frequency of different clonal complexes (Table 2). In GBS from human hosts, CC19-19 was most prevalent (35%), followed by CC23 (20%). In contrast, GBS strains isolated from ruminants were most commonly assigned to CC23 (21%), strains from dogs to CC19-10 (40%), strains from rodents to CC19-01 (75%), and strains from fish to CC260/261 (75%). Some host-specific differences were also visible in the prevalence of capsular serotypes (Table 3). While serotype IB was highly prevalent in GBS strains from fish (63%), it was only rarely detected in isolates from other hosts. In contrast, serotypes IA, II, III, and V were common in GBS from different host species. As illustrated in the SplitsTree (see Fig. 2), the *S. agalactiae* strains investigated in this study also exhibited highly heterogeneous DNA microarray hybridization profiles. With the exception of *S. agalactiae* isolated from fish, no distinct clustering of strains based on host species, geographical origin, or clonal complex assignment could be observed.

191 The prevalence of selected virulence and resistance genes among different host groups
192 is presented in Table 4. Depending on the host, different combinations and variants of the
193 pilus island gene clusters were observed. The *speM* gene encoding exotoxin M was detected
194 in only one isolate (S209, CC19-19), originating from a recto-vaginal swab from a patient in
195 China. With regard to the allelic variants of the alpha-like GBS surface proteins, the allele
196 *alp_rib (R4)* was significantly more prevalent in strains of human origin than in strains from
197 all other sources. The *bac* gene encoding a GBS surface protein was frequently present in
198 isolates from dogs. In addition, the genes of the first pilin gene cluster (*pilA/B/C-I*) were more
199 common in canine GBS isolates, whereas prevalence was low in fish isolates. In contrast, the
200 *pilA/B/C-2b* genes of the second pilin gene cluster were significantly more prevalent in GBS
201 from fish compared with GBS isolated from humans, dogs, and rodents. The vast majority of
202 human isolates (94%) harbored *scpB*, which encodes for C5a peptidase and is used as a
203 diagnostic marker.
204 As for genes conferring resistance to antimicrobial agents, the *emrB/qacA* multidrug
205 resistance transporter gene was present in all tested strains. The majority of strains also
206 exhibited *tetM,* a gene associated with tetracycline resistance, and *cadD,* involved in cadmium
207 resistance. Among human and canine strains, we frequently detected *merA/R,* genes involved
208 in mercuric resistance. Online Resource 2 provides a comprehensive overview of the
209 frequency of all virulence and resistance genes detected among the different host groups, as
210 well as *p*-values for statistically significant differences. Full DNA microarray hybridization
211 patterns of all strains are included in Online Resource 3.
212
213 **Discussion**
214 To date, data on GBS interspecies transmission is limited. In particular, the zoonotic
215 potential and the directionality of transmission of GBS infections are poorly understood.

Experimental studies showed the transmissibility of various bovine and human GBS strains to fish [26–28] and characterization and genotyping studies suggested occasional transmission between humans and cattle [23,24]. Very recently, transmission of *S. agalactiae* through ingestion of raw fish sushi was reported to have led to severe infections in humans (Kalimuddin et al., 2017). In addition, cases of GBS infections acquired through contact with GBS from other host species have been reported: necrotizing fasciitis and endocarditis cases in humans occurred after a dog [25] and a cat bite [21], respectively, and necrotizing fasciitis cases in a group of crocodiles were likely of human origin [22].

In our study, isolates from various hosts were assigned to the same strain, suggesting interspecies transmission. Five GBS strains were detected in at least one bovine and one human host, and another strain was detected in a human, a bovine, and two canine hosts. In addition, a canine and a porcine isolate were assigned to the same strain. The relatively high number of *S. agalactiae* strains identified in both a sample from a dog and at least one other host species is particularly striking, considering that only 15 canine strains were included in this study.

Nitschke et al. [13] introduced GBS typing based on DNA microarray hybridization patterns and provided data on human GBS from Germany and the Caribbean, as well as bovine GBS from Germany: The most prevalent hybridization patterns detected were HP-01 (CC19-01), HP-30 (CC19-17), HP-35 (CC19-19), and HP-48 (CC23), corresponding to the whole-genome sequenced reference strains CJB111, COH1, Gottschalk 1003A, and Strain 515, respectively . All four hybridization patterns were also frequently detected in our study, with HP-01 being linked to the most diverse set of hosts. GBS of HP-01 originated from humans (n = 5), cows (n = 3), dogs (n = 2), mice (n = 3), emerald monitors (n = 2), a rat (n = 1), and a snake (n = 1). GBS of HP-30 originated from human hosts (n = 10), a rabbit (n = 1), a cow (n = 1), and a goat (n = 1). GBS of HP-35 originated from humans (n = 8), a dog (n =

241 1), and a cow (n = 1), and GBS of HP-48 were detected in human (n = 15), bovine (n = 3),
242 and canine (n = 2) hosts.
243 The versatility and wide spread of these strains becomes evident, when considering the hosts
244 and geographical locations, in which some of the strains investigated in this study were
245 isolated: S60/S250/S256 (HP-01) was detected in a sample from the skin of a dog in Germany,
246 as well as in a human vaginal swab from China, and bovine mastitis milk in Germany.
247 S117/S254 (HP-30) was identified in a sample from a rabbit in Germany, as well as in human
248 samples in Germany and Colombia. S185/S255 (HP-35) was detected in a sample from the
249 paw of a dog in Germany, and vaginal swabs from women in Colombia and Switzerland.
250 This study provides comprehensive data on the occurrence of capsular serotypes
251 among human and animal GBS isolates. CPS typing data is not only essential for
252 epidemiological purposes, but is also needed in the development of effective CPS-based
253 vaccines [11,12,32].
254 Among the GBS strains investigated in this study, we frequently detected genes
255 conferring resistance to antimicrobial agents and heavy metal resistance markers. Genes
256 associated with macrolide/ clindamycin resistance were exclusively found among GBS from
257 humans, ruminants, dogs, and a pig. Various recent studies report that 15-21% of GBS strains
258 isolated from pregnant women or cases of neonatal GBS infections are resistant to macrolide
259 and/or lincosamide [33–35]. The high prevalence of *tetM* detected in our study in human
260 (76%) and ruminant (48%) strains is consistent with findings of Nitschke and colleagues,
261 which reported prevalence rates of 78% and 71% in human GBS from Germany and the
262 Caribbean, as well as 48% in bovine GBS from Germany [13].
263 In our study, 40% of the canine strains and 25% of fish strains exhibited *bac,* while the
264 gene was only detected in 13% of GBS strains from human origin. The *bac* gene encodes the
265 C protein beta antigen (Bac), which is able to simultaneously bind to the Fc fragment of IgA

266 and the complement regulator factor H, thus likely contributing to immune evasion [32,36]. In
267 addition, increased Bac expression was reported in invasive strains compared to strains
268 collected from vaginal carriers [37]. Previous studies have associated *bac* sequence types with
269 capsular serotype assignment [37,38]. In contrast to our findings, a study investigating human
270 GBS from Asia, Australia, Europe, New Zealand, and North America found that *bac* was
271 present in 97% of serotype Ib isolates and 37% of serotype II isolates, while being largely
272 absent in GBS assigned to other serotypes [38].

273 Low prevalence of the *speM* gene encoding exotoxin M has been reported among GBS
274 from human and bovine sources [13]. This is consistent with our findings. In this study, we
275 detected *speM* in only one isolate (S209, CC19-19) originating from a recto-vaginal swab
276 from a patient in China.

277 In our study, the alpha-like GBS surface protein allele *alp_rib (R4)* (= R4, rib) was
278 significantly more prevalent in strains of human origin than in strains from all other sources.
279 The alpha-like proteins are chimeras forming mosaic structures on the surface of the organism
280 [39]. While the function of many alpha-like proteins is still poorly understood, they may act
281 as invasins mediating adherence to cervical epithelial cells, as well as transmembrane passage
282 and translocation of the organism [39].

283 In our study, different hosts were associated with different combinations and allelic
284 variants of genes of the pilus islands. Each of the three pilus islands (Pl-1, Pl-2a, Pl2b)
285 encodes one backbone and two ancillary proteins that mediate interactions with host cells. The
286 pilus islands and their combinations were shown to play an important role in host adaptation
287 and specificity, as well as disease presentation [40].

288 The findings of our study suggest that while *S. agalactiae* is well adapted to various hosts
289 including humans, cattle, dogs, rodents, and fish, interspecies transmission is possible and
290 occurs amongst others between humans and cows, dogs, and rabbits. Involvement of a canine

host in interspecies transmission events may be particularly frequent, with the directionality of transmission still being unclear. The virulence and resistance gene patterns determined in our study significantly extend the limited current knowledge on interspecies transmission. They could also be utilized in the identification of suitable targets for therapeutic agents, as well as vaccines.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical Approval

This study was carried out in accordance with ethical clearance and informed consent regulations of the locally cognizant ethics commission. All isolates were part of existing strain collections with anonymized sample information. No animal or human hosts were subjected to sampling for the purpose of the present study.

Informed consent

This was a retrospective study. For this type of study formal consent is not required.

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426 **Figure legends**

427

428 **Fig 1 Interspecies transmission.** Several *S. agalactiae* strains were detected in samples from
429 more than one host species, indicating interspecies transmission. This figure provides an
430 overview of the links detected and their frequency.

431

432 **Fig 2 SplitsTree.** SplitsTree illustrating the degree of similarity of virulence and resistance
433 gene profiles of *S. agalactiae* strains from different sources: Human host (pink), ruminant
434 (green), dog (orange), elephant (grey), fish (blue), rodent/rabbit (yellow), other (purple).
435 Strains detected in two or more host species are marked by red circles.

436

437 **Tables**

438

439 **Table 1: Clonal lineages and strains identified in more than one continent and across**
440 **multiple host species.** In some clonal complexes, strains were isolated more than once, some
441 of them beyond country borders and from different host species.

Clonal complex	Strain	Source	Sample	Country[a]
CC19-01	S48/S244/S245	Rat (n = 1)	Abscess	CH
		Monitor (n = 2)	Lung/ kidney/ liver/ intestine	DE
		Mouse (n = 3)	Intestine	DE
	S53	Mouse (n = 5)	Intestine	DE
	S57/ S249	Snake (n = 2)	Liver, skin	DE
		Monitor (n = 1)	Liver	DE
	S60/S250/S256	Dog (n = 2)	Skin	DE
		Human (n = 4)	Vaginal swab	HK
		Bovine (n = 2)	Milk	DE
	S61/S251	Rat (n = 1)	Trachea	DE
		Mouse (n = 2)	Prepuce	DE
	S63	Bovine (n = 4)	Milk	DE
	S64	Bovine (n = 3)	Milk	DE
	S65	Bovine (n = 2)	Milk	DE
	S69	Bovine (n = 5)	Milk	DE
	S81	Human (n = 2)	Vaginal swab	HK
	S84	Human (n = 2)	Vaginal swab	HK

	S92	Human (n = 2)	Vaginal swab	HK
	S102	Human (n = 2)	Vaginal swab, abdominal tissue	HK, KY
CC19-02	S3	Guinea pig (n = 2)	Nose, liver	DE
	S7	Bovine (n = 2)	Milk	DE
CC19-10	S58/S252	Bovine (n = 1)	Organs	DE
		Human (n = 3)	Urine, vaginal swab, wound	CO, CH, KY
	S66	Bovine (n = 2)	Uterus, milk	DE
	S68	Bovine (n = 3)	Milk	DE
	S73	Human (n = 2)	Pus, urine	CO
	S85	Human (n = 2)	Vaginal swab	HK
	S90	Tilapia (n = 4)	Kidney	TH
	S91	Tilapia (n = 2)	Kidney	TH, VN
	S112	Human (n = 2)	Urine, blood	KY
CC19-17	S116	Human (n = 4)	Mastitis, blood, vaginal swab	DE, CO, CH
	S117/S254	Rabbit (n = 1)	Unknown	DE
		Human (n = 4)	Vaginal swab, urine	CO, CH
	S120	Elephant (n = 3)	Abscess/ foot	DE
	S126	Bovine (n = 2)	Milk	DE
	S152	Human (n = 3)	Vaginal swab	HK
	S153	Human (n = 2)	Vaginal swab	CH
	S157	Human (n = 2)	Vaginal swab	CH
	S169	Human (n = 4)	Vaginal swab	CH
	S175	Human (n = 4)	Blood, urine, vaginal swab	KY

CC19-19	S186/S255	Dog (n = 1)	Paw	DE
		Human (n = 4)	Vaginal swab, urine	CO, CH
	S190	Human (n = 2)	Urine, vaginal swab	CO
	S193	Human (n = 2)	Urine	CO
	S195	Human (n = 3)	Urine, vaginal swab	CO
	S197	Human (n = 2)	Vaginal swab	CO
	S198	Human (n = 2)	Urine, blood	CO
	S218	Human (n = 2)	Vaginal swab	CH
	S222	Human (n = 2)	Vaginal swab	CH, KY
	S227	Human (n = 3)	Vaginal swab	CH
	S235	Human (n = 2)	Vaginal swab	CH
	S237	Human (n = 2)	Blood, vaginal swab	KY, CO
CC19-67	S5/S243	Dog (n = 1)	Skin	DE
		Bovine (n = 1)	Milk	CH
	S17	Bovine (n = 4)	Milk	CO
	S23	Bovine (n = 2)	Milk	CH
CC23	S124/248	Dog (n = 1)	Skin	DE
		Pig (n = 1)	Milk	DE
	S128	Bovine (n = 2)	Milk	DE
	S130	Bovine (n = 2)	Milk	DE
	S133	Bovine (n = 3)	Milk	DE
	S134/S253	Bovine (n = 1)	Milk	DE
		Human (n = 3)	Urine, vaginal swab	CO, HK
	S135	Bovine (n = 2)	Milk	DE

	S137	Human (n = 12)	Vaginal swab, biopsy, urine, blood	CO, CH, KY
	S139	Human (n = 3)	Urine, blood, secretion	CO
	S141	Human (n = 2)	Vaginal swab, urine	CO
	S142	Human (n = 2)	Vaginal swab	CO
	S145	Human (n = 3)	Vaginal swab	CH, CO
	S162	Human (n = 3)	Vaginal swab	CH
CC103	S11/S247	Bovine (n = 1)	Milk	DE
		Human (n = 1)	Pus	CO
	S14	Bovine (n = 5)	Milk	DE
	S16/S246	Bovine (n = 1)	Milk	DE
		Human (n = 1)	Urine	CO
CC260/261	S31	Tilapia (n = 2)	Spleen, kidney	HN, CO
	S32	Tilapia (n = 3)	Spleen, kidney	HN, CO
CC298	S19	Bovine (n = 3)	Milk	CO
not assigned	S10	Bovine (n = 2)	Milk	DE
	S18	Bovine (n = 2)	Milk	CO

[a] Country abbreviations: CH = Switzerland, CO = Colombia, DE = Germany, HK = Hong Kong SAR (China), HN = Honduras, KY = Kenya, TH = Thailand, VN = Vietnam

449 **Table 2. Clonal complex distribution.** This table provides an overview of the prevalence of
450 different clonal complexes among *S. agalactiae* strains from various hosts (in percent).

Clonal complex	**Hosts (% of strains)**					
	Human (n = 161)	**Ruminant (n = 52)**	**Dog (n = 15)**	**Rodent (n = 8)**	**Fish (n = 8)**	**Other (n = 12)**
CC19-01	12	19	13	75	0	25
CC19-02	4	2	7	25	0	0
CC19-04	1	0	0	0	0	0
CC19-10	12	12	40	0	25	17
CC19-17	10	6	0	0	0	17
CC19-19	35	4	13	0	0	0
CC19-22	2	0	0	0	0	0
CC19-67	1	13	7	0	0	8
CC23	20	21	20	0	0	33
CC26	1	0	0	0	0	0
CC103	2	10	0	0	0	0
CC130	1	0	0	0	0	0
CC260/261	0	0	0	0	75	0
CC298	0	2	0	0	0	0
not assigned	0	12	0	0	0	0

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452 **Table 3: Prevalence of capsular serotypes.**

Capsular serotype	**Hosts (% of strains)**					
	Human (n = 161)	**Ruminant (n = 52)**	**Dog (n = 15)**	**Rodent (n = 8)**	**Fish (n = 8)**	**Other (n = 12)**
IA	16	35	20	0	13	25
IB	9	8	7	0	63	0
II	17	19	20	50	0	0
III	22	21	13	0	13	33
IV	4	10	13	0	0	0
V	21	8	20	50	0	25
VI	1	0	0	0	0	0
VII	2	0	0	0	0	0
IX	1	0	0	0	0	0
negative	2	0	0	0	13	17
not assignable	5	0	7	0	0	0

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Table 4. Virulence and resistance genes. Prevalence of selected virulence and resistance genes among GBS strains isolated from different hosts: humans, ruminants, dogs, rodents, fish, and other (snake, turtle, frog, elephant, pig, rabbit). A comprehensive list of DNA microarray results including *p*-values is provided as Supplementary Table 2.

Gene	**Function**	**Host (% of strains)**					
		Human (n = 161)	**Ruminant (n = 52)**	**Dog (n = 15)**	**Rodent (n = 8)**	**Fish (n = 8)**	**Other (n = 12)**
Virulence genes							
speM	Exotoxin M	1	0	0	0	0	0
cylD	Beta hemolysin locus	96^{*F}	100^{*F}	100^{*F}	100^{*F}	25^{*HRDXY}	100^{*F}
cylE	Beta hemolysin locus	87^{*F}	94^{*F}	100^{*F}	100^{*F}	25^{*HRDXY}	100^{*F}
alp_3	Allele of the α-like protein/ α-antigenic cell wall protein	7^{*X}	10^{*X}	13^{*X}	75^{*HRDF}	0^{*X}	25
alp_rib (R4)	Allele of the α-like protein/ α-antigenic cell wall protein	52^{*RXFY}	24^{*H}	20	0^{*H}	0^{*H}	9^{*H}
bac	β-antigenic cell wall protein	13^{*D}	15	40^{*H}	0	25	8
pilA1	Pilin gene cluster 1	51	50	80^{*F}	71	25^{*D}	50

pilB1	Pilin gene cluster 1	63	48*D	80*RF	75	25*D	50
pilC1	Pilin gene cluster 1	66	56	80	75	38	50
pilA2a	Pilin gene cluster 2a	81*RFY	52*HDX	87*RF	100*RFY	13*HDX	50*HX
pilC2a	Pilin gene cluster 2a	82*RF	52*HDX	93*RF	100*RF	13*HDX	58
pilA2b	pilin gene cluster 2b	15*RFY	48*HDX	7*RF	0*RF	63*HDX	42*H
pilB2b	Pilin gene cluster 2b	15*RFY	48*HDX	7*RF	0*RF	67*HDX	42*H
pilC2b	Pilin gene cluster 2b	14*RFY	48*HDX	7*RF	0*RF	75*HDX	42*H
scpB-var1	Complement-inactivating C5a peptidase	94*RDXFY	50*H	67*HF	25*H	13*HD	27*H
scpB-var2	Complement-inactivating C5a peptidase	94*RDXFY	48*H	67*HF	25*H	13*HD	25*H
fsb-var3	Allele of a fibrinogen binding protein	61*X	46*X	73*F	100*HRFY	25*DX	33*X
Resistance genes							
cadC	Cadmium efflux system accessory protein	21*R	2*H	13	0	0	0
cadD	Cadmium resistance protein	75*F	77*F	93*FY	100*FY	14*HRDX	50*DX
emrB/qacA	Multidrug resistance transporter	100	100	100	100	100	100
ermA	Macrolide/clindamycin resistance	9	2	13	0	0	8

ermB	Macrolide/clindamycin resistance	19	10	7	0	0	0
ermC	Macrolide/clindamycin resistance	0	0	0	0	0	0
merA	Mercuric reductase	58^{*RXFY}	11^{*HD}	45^{*R}	0^{*H}	13^{*H}	10^{*H}
merR	Mercuric resistance operon regulatory protein	57^{*RXFY}	12^{*H}	33	0^{*H}	13^{*H}	17^{*H}
tetM	Tetracycline resistance	76^{*RF}	48^{*HD}	93^{*RF}	75	25^{*HD}	58

*The distribution of the respective gene differed significantly between strains from the stated hosts (with $p \leq 0.050$). Host groups are indicated as follows: humans (H), ruminants (R), dogs (D), rodents (X), fish (F), and other (Y).

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