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Prevalence, risk factors and transmission dynamics of ESBL-producing *Enterobacteriaceae*: a national survey of cattle farms in Israel, 2013

Amos Adler[1*], Na'ama Sturlesi[2], Noga Fallach[1], Deniz Zilberman-Barzilai[1], Omar Hussein[1], Shlomo E Blum[3], Eyal Klement[2], Mitchell J Schwaber[1] and Yehuda Carmeli[1].

[1]National Center of Infection Control, Ministry of Health, affiliated with the Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel

[2]Koret School of Veterinary Medicine, Hebrew University, Rehovot, Israel

[3]Kimron Veterinary Institute, Beit Dagan, Israel

***Corresponding author email address: amosa@tlvmc.gov.il**

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Abstract

Our objectives were to study the prevalence, risk factors for carriage and transmission dynamics of ESBL-producing Enterobacteriaceae (ESBLPE) in a national survey of cattle. This was a point-prevalence study conducted from July to October 2013 in Israel. Stool samples were collected from 1226 cows in 123 sections in 40 farms of all production types. ESBLPE were identified in 291 samples (23.7%): 287 *E. coli* and 4 *K. pneumoniae*. The number of ESBLPE-positive cows was highest in quarantine stations and fattening farms and was lowest in pasture farms (p=0.03). The number of ESBLPE-positive cows was lowest in sections containing adult cows (>25 months) and highest in calves (<4 months) (p<0.001). Infrastructure variables that were significant risk factors for ESBLPE carriage included crowding, lack of manure cleaning and lack of a cooling (p<0.001 for each) all of which were more common in calves. Antimicrobial prophylaxis was given almost exclusively to calves and was associated with a high number of carriers (p<0.001). The 287 *E. coli* isolates were typed into 106 REP-PCR types, harboring mostly $bla_{\text{CTX-M-1}}$ or $bla_{\text{CTX-M-9}}$ group genes. The six farms with ≥15 isolates of ESBLPE had 4-7 different REP-PCR types with one dominant type present in about half of the isolates. Fourteen types were identified in more than 1 farm, with only 6 of the farms adjacent to each other.

The prevalence of ESBLPE carriage is high in calves in cowsheds where the use of antimicrobial prophylaxis is common. ESBLPE disseminate within cowsheds mainly by clonal spread, with limited inter-cowshed transmission.

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Introduction

Since the advent of the first antimicrobials, antimicrobial resistant bacteria (AMRB) have spread in conjunction with the use of their respective antimicrobial agents (1). Accordingly, the emergence of extended-spectrum β-lactamase (ESBL) enzymes as a global threat followed the introduction of third-generation cephalosporins (TGC), used mainly in healthcare settings (2). Although the ESBLs were first noted in the 1980's, a substantial increase in their prevalence in *Escherichia coli* was noted in the 2000's. This increase was related mainly to the emergence of a pandemic clone, designated Sequence Type (ST) 131. A particular worrisome feature of this clone was its predominance in community-onset cases of infection with ESBL-producing bacteria (3). This epidemiologic feature has attracted attention to the possibility of sources of acquisition other than healthcare settings, including the food and livestock industries. Indeed, many studies have documented the presence of ESBL-producing bacteria in a variety of meats and other livestock-origin food samples. Although the use of antimicrobials has often been suggested as the main culprit for this phenomenon (4), there are in fact no studies that have looked into this question. Moreover, the data regarding other risk factors for carriage of ESBL-producing *Enterobacteriaceae* (ESBLPE) in livestock in general and cattle in particular is limited to the analysis of only a few factors (5, 6). Also, although molecular analysis of these isolates has been reported, such data have never been used for the analysis of the dissemination dynamics of ESBLPE in and between farms. In Israel, there is a large industry of cattle farms for both dairy and meat products. Despite that, there are no data regarding the prevalence of AMRB in cattle in Israel. The objectives of this work were to study the prevalence of ESBLPE carriage among cattle in a nationwide

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survey in Israel, to analyze the dissemination dynamics of these strains using molecular studies and to analyze the risk factors for carriage.

Methods:

Study design and data collection

This was a point-prevalence study conducted from July to October 2013 in cattle farms from the main farming locations in Israel (figure 1). The study included 1226 cows placed in 123 sections on 40 farms of all types: dairy, fattening (both representing intensive farming without grazing), pasture and mixed (intensive dairy and fattening) farms. Farms are typically divided to separate sections according to age groups and sampling was done accordingly. It also included two quarantine stations that hold imported calves prior to their transfer to fattening farms. The study included stool and data collection (see below). As cows are typically separated inside the farms according to age, sampling was done from approximately 10 heads at each section: dairy farms-4 sections; fattening- 2-3 sections; pasture farms-calves, adult females and bulls. Sampling was done individually by rectal sampling (mainly in calves) or from freshly excreted manure and was delivered directly to the laboratory of the National Center for Infection Control.

Data were collected by a single author (NS), by direct observation or by questioning of the farm's manager, and included variables pertaining to the section or the entire farm. The section-related variables included age, crowdedness (head/m^2), animal cleanliness (graded as the percentage of clean animals in the section), environmental cleanliness, infrastructure variables and use of antimicrobial prophylaxis. The farm-

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related variables included geographical location, farming type, recent introduction of new calves and variables related to veterinary care.

Microbiological and molecular methods

Stool (~1 gr) was inoculated in BHI broth and incubated overnight at 36 ºC. A broth aliquot of 10 µl was subcultured onto CHROMAgar ESBL™ agar plates (Hylabs, Rehovot, Israel) and incubated overnight. Suspicious colonies were identified according to the manufacturer's instructions. Identification was done using the ENTEROTEST™ kit with a Citrate test (Hylabs, Rehovot, Israel) and the VITEK-2 system (bioMérieux, Marcy l'Etoile, France) in equivocal cases. ESBL testing was done by the combined disk method using ceftazidime and cefotaxime disks alone and with clavulanic acid. Antimicrobial susceptibility testing (AST) was done by disk diffusion and interpreted according to CLSI criteria (7); susceptibility to colistin was determined by initial screening via disk diffusion followed by MIC testing via gradient method (Etest®, bioMérieux, Marcy l'Etoile, France) for isolates yielding a disk diffusion diameter of less than 10 mm.

Molecular typing was done by repetitive extragenic palindromic (REP)-PCR (8) or BOX-PCR (9) for ESBL-producing *E. coli* and *Klebsiella pneumoniae*, respectively. PCR products were resolved using capillary-gel electrophoresis apparatus (QIAxcel, QIAGEN, Hilden, Germany) and visually compared; isolates with an identical pattern were regarded as one strain. An example of this comparison is presented in figure S1. The bla_{ESBL} gene was determined by PCR for the $bla_{\text{CTX-M}}$ group (10) and by PCR and sequencing for the bla_{TEM} and bla_{SHV} alleles (11).

Statistical analysis

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With the exception of the microbiological data, all other data (non-dependent variables) were collected per sections and farms but not for individual cows. Continuous parameters are presented by mean and standard deviation (SD) of the variable and include the number of non-missing values. Categorical variables presented are the number and percentages of the sections in each category and the number (+/-SD) of the positive ESBLPE carriers in each category.

Multiple imputation of missing data was conducted, but bivariate analyses with the imputed data for the relevant covariates did not converge; therefore all analyses were conducted with available data only. Bivariate analyses were conducted with mixed Poisson regression models for each covariate separately, with a random effect of farms and an offset of number of units within section. The prevalence ratio (PR) and the p-values are presented. A p-value of less than 0.05 was considered statistically significant. Multivariable analyses were conducted including all covariates with p-values (from the bivariate analysis) ≤ 0.1, excluding those covariates with missing data. Because of the pronounced differences in living conditions, analyses were done separately for non-pasture farms, in addition to the analysis for all farms. Statistical analyses were conducted with SAS© version 9.2.

Results

Farming type, location and of prevalence of ESBLPE in Israeli farms

The study included 1226 cows placed in 123 sections on 40 farms, the majority of which were dairy farms (table 1). The number of cows sampled was 10 in 109 sections (including all pasture sections) and from 9 to 11 in an additional 115/123

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sections (93.4%). Hence, the prevalence in the sections is represented by the number of positive ESBLPE carriers (tables 1 and 2). Overall, ESBLPE were identified in 291 cows (23.7%), and prevalence was highest in the quarantine stations and fattening farms and lowest in pasture farms (table 1). The farm type was not significant in multivariate analysis (PR=0.053 for pasture farms, p=0.079). The locations of the farms according to farming type are presented in figure 1. Dairy farms were located across the country and especially in the Western Negev area (arid to semi-arid climate), whereas pasture farms were mainly in the Jezreel valley and the Golan Heights (Mediterranean climate). Geographical location was not correlated with the prevalence of ESBLPE carriage (data not shown).

Risk factors for ESBLPE carriage

Descriptive statistics and bivariate analysis of the risk factors for ESBLPE carriage are presented in table 2. The variables are presented in three groups: infrastructure and cleanliness related, veterinary treatment related and farm related. Variables defined by distinct groups, including age and farm type, are further discussed below.

Variables related to infrastructure and cleanliness: lack of a cooling system, increased crowdedness and lack of manure cleaning were all significantly associated with increased risk for ESBLPE carriage (table 2). Cooling with nebulizers (in addition to fans) and manure cleaning using slatted floors were associated with the lowest risk. In multivariable analysis, only the use of fans with nebulizers (PR=0.2211, p=0.036) was identified as significant factor protecting against ESBLPE carriage. Unexpectedly, the degree of cleanliness, both of cows and of water troughs, was related with ESBLPE carriage. A low number of cows per section was associated with increased risk for ESBLPE carriage, likely related to specific risk groups (see below).

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Variables related to veterinary treatment.

Antimicrobial prophylaxis was associated with increased risk for ESBLPE carriage in the bivariate analysis ($p<0.001$). It was administered in 33 sections overall, on all farm types, most commonly (n=26) in calves (<4 months). The most common agents were tetracycline (n=26, 69%), either as chlortetracycline or doxycycline. Other agents included norfloxacin (n=4), cephalosporin agents (cephalexin or ceftiofur, n=4), anti-coccidiosis agents (n=3), sulfa agents (n=3), gentamicin (n=1) and monensin (n=1). In eight sections more than one agent was given, most commonly in addition to a tetracycline. ESBLPE carriage increased with increased frequency of veterinarian visits; vaccination with more than the mandatory vaccines was not associated with increased risk.

Variables related to farm.

The arrival of new cattle in the preceding month occurred in 26% of the sections, mostly from other Israeli farms, and was not associated with increased risk for carriage. Similarly, the geographical distribution of the farms (figure 1) was not associated with increased risk (table 2).

Farming groups associated with increased risk for ESBLPE carriage

The mean prevalence of ESBLPE carriage was highest in calves (PR=5.3, SD=3.6) and declined gradually in adult cows (PR=0.9, SD=1.4). This pattern was apparent in all farm types (figure 2) but was most pronounced in dairy farms. This suggests, as growing calves are transferred to the next age-groups, that ESBLPE carriage is acquired mostly in calves and is lost gradually with maturation. Hence, we compared the most relevant risk factors between the age groups. Compared with the other age

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groups, calves had by far the highest use of antimicrobial prophylaxis (78.6 vs. 7.7 %). Due to the vast difference in living conditions and physiology, a comparison of these factors in calves compared to older cows was problematic. For example, calves lived in the most crowded conditions (67.9% lived in less than 5 m^2 per head vs. 5.5% in other age-groups). However, since calves on dairy farms are usually placed in individual pens, the relevance of these factors to ESBLPE carriage on these farms is less clear.

As presented above (table 1), different farm types varied significantly in the prevalence of ESBLPE carriage. However, although the distribution of age groups was similar overall, it differed widely on different farms (figure 3), thus affecting the overall prevalence of ESBLPE. For instance, quarantine stations included calves only and consequently had the highest prevalence. On the other hand, pastures did not include calves younger than 4 months, and thus had the lowest prevalence. The latter had very different living conditions and hence the comparisons of crowdedness, manure cleaning and cooling were irrelevant.

The cross comparison of age and farm types, have identified the calves' (<4 months) sections in the dairy farms as those with the highest prevalence of ESBLPE carriage (figure 3). We therefore compared these sections to the other sections in the dairy farms as well as to the calves' sections in the fattening farms (table 3). Compared with both of these groups, calves' sections in dairy farms were more crowded (albeit with separate pens), had no cleaning of manure and received antimicrobial prophylaxis almost universally. Use of cooling was more common in non-calves' sections in dairy farms.

Antimicrobial resistance patterns and molecular characteristics of ESBLPE isolates

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The majority of isolates were ESBL-producing *E. coli* (ESBLEC, n=287, 98.6%) and the rest (n=4) were *K. pneumoniae*. Non-susceptibility to other antimicrobial agents was found as follows: tetracycline- 267 (91.7%), trimethoprim-sulfamethoxazole-233 (80%), streptomycin-123 (42.2%), chloramphenicol-108 (37.1%), ciprofloxacin-72 (24.7%), gentamicin-58 (19.9%) and amoxicillin-clavulanate-58 (19.9%). All isolates were susceptible to ertapenem, colistin, nitrofurantoin and fosfomycin.

The most common bla_{ESBL} gene was bla_{CTXM-1} group (n=233, 80%), followed by bla_{CTXM-9} group (n=28, 9.6%) and bla_{SHV-12} (n=24, 8.2%). No bla_{ESBL} gene was identified in 6 isolates that tested positive by the ESBL combined disk test.

The 287 *E. coli* isolates were typed by REP-PCR into 106 different types, of which 55 were singletons and 19 were common to two isolates. Monoclonal spread was not limited to a single farm: of the 26 types identified in ≥4 isolates, 16 types were identified in 1 farm only, 8 in 2 farms and 2 in 3 farms. The 3 most frequent types were identified in 20 isolates (8 sections and 3 farms), 13 isolates (5 sections and 2 farms) and 11 isolates (3 sections in 3 farms), respectively. However, the route of dissemination was not apparent in most cases: of the 14 types that were found in more than one farm, geographical proximity was apparent in only 6 types, all of them in dairy farms in the Western Negev area. Also, there were no types shared between calves on the quarantine station and fattening farms, the latter being the usual destination of these calves.

The farm with the largest number of ESBLEC (farm 12, n=23) showed a diverse clonal structure with one dominant type (n=12) and three additional types. All four types were present in the ESBLPEC isolates retrieved from suckling calves but only one or two types were identified in the other sections (figure 3). The other five farms

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with ≥15 isolates of ESBLEC per farm had 6-7 different REP-PCR types identified at each farm with one dominant type present in up to half of the isolates. Similar to farm 12, a diverse population of types was represented in the isolates retrieved from suckling calves.

The four ESBL-producing *K. pneumoniae* were divided into 3 BOX-PCR types that were present on 3 farms.

Discussion

Surveillance of AMRB's in livestock in general and cattle in particular, is typically done from an anthropocentric perspective, and thus it is focused mainly on their implications in humans, via either the food chain or the environment (12, 13). Thus, there are only a few studies that have looked into the prevalence and risk factors for ESBLPE carriage in cattle (5, 6). A valid comparison of prevalence and risk factors for ESBLPE carriage between countries is very difficult even in human studies, due to the vast differences in the populations and the methodology of the different studies (2). Such comparisons are probably even more problematic in veterinary studies that involve widely varying designs and methodology, such as the sampling site and the type of cattle included in the survey. With that in mind, it is hardly surprising that the prevalence found in our study (23.7%) was very different than the prevalence found in the Bavarian or the Swiss studies (32.8% and 8.4%, respectively) (5, 6). A major difference relates to the sampling site that was located in the farms in both our study and the Bavarian study, whereas in the Swiss study the samples were collected at the slaughterhouse. This difference by itself has tremendous implications on the population selected in regard to both the animal age and farming practices and may explain the lower prevalence found in the Swiss study. Wider comparison based on

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the routine surveillance programs undertaken in several European countries (14) are even more problematic. In these programs, *E. coli* isolates (one per epidemiological unit) are picked randomly at the slaughterhouse and the burden of resistance is measured by the proportion of resistant isolates among those picked. Clearly, this methodology risks underestimating the actual prevalence, a likely explanation for the relatively low proportions of cefotaxime-resistant isolates (representing ESBL producers) found in these reports (e.g., 2.5% in Germany).

The highest prevalence of ESBLPE was found in the youngest age groups, usually suckling calves, and this declined gradually with increasing age, as much as 6.5-fold in adult cows. This finding is similar to previous studies in cattle (5, 6). Also, it is reminiscent of other AMRB surveillance studies (e.g., methicillin-resistant *Staphylococcus aureus*) that were done in human infants in the first two years of life, that demonstrate a pattern of early acquisition followed by a gradual decline in prevalence (15). This proposed paradigm is supported by the molecular typing analysis, which showed a diversity of strains in the youngest age group of a particular farm. Later, these strains were partially presented in the older age groups, with occasional spread inside these groups (figure 3).

Methodologically, this observation (early acquisition of ESBLPE followed by gradual decline) suggests that a longitudinal design would have been more appropriate for risk factor analysis than the point prevalence design used in our study. Hence, we think that the bivariable and multivariable analyses that were done on the entire population might have been misleading in regard to some of the risk factors identified. For instance, the higher proportion of calves sampled in fattening farms compared with dairy farms explains the overall higher prevalence of ESBLPE in the former (table 1), despite the higher prevalence of carriage at each of the age groups in the latter (figure

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2). Therefore, we performed subgroup analyses (table 3) that allowed partial compensation for the basic limitation of the study design.

From these analyses, it seems that the most conspicuous factor distinguishing calves from the other groups is the use of antimicrobial prophylaxis. The most commonly used agents were tetracyclines (rather than cephalosporins), suggesting a connection between the use of these agents and ESBLPE carriage. Such a connection is not surprising, as there have been numerous reports correlating the use of agents other than cephalosporins (e.g., quinolones) with infections or carriage of ESBLPE (2). This finding can be explained by the overall effect of broad-spectrum antimicrobials on the gut microbiota or by the presence of different resistance genes on a shared mobile element (16). Indeed, the rate of tetracycline resistance in these isolates was extremely high - 91.7%. Although it is beyond the scope of this study to perform a risk-benefit analysis of the use of prophylaxis in this age group, this seems to be the most important factor that can be targeted for intervention. It is noteworthy that from an anthropocentric perspective, the decline in the prevalence of ESBLPE with age suggests a decreased potential for transmission of ESBLPE to humans.

In addition to antimicrobial prophylaxis, these comparisons also highlighted the importance of several infrastructure factors, including crowdedness and lack of cleanliness, as associated with increased prevalence of ESBLPE carriage. Unfortunately, we were unable to demonstrate these associations conclusively in multivariable analysis due to the basic shortcoming of the design.

Although many studies have included molecular analysis of ESBLPE isolates from cattle, our study is unique in its use of typing data for the understanding of transmission dynamics in this population. In addition to its contribution to our

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understanding of intra-farm dissemination (discussed above), this analysis demonstrated the presence of identical isolates in different farms, most importantly in neighboring farms. The *bla*$_{ESBL}$ gene analysis showed a predominance of the *bla*$_{CTX\text{-}M\text{-}1}$ group, similar to previous studies. Along with the heterogeneity of the isolate population, the predominance of a single group suggests that horizontal gene transfer also plays an important role in the dissemination of these genes, as was also found to be the case with other resistance genes in *E. coli* (17).

In conclusion, despite the design-based limitations of this study, we were able to provide a combined epidemiological and molecular hypothesis for the dissemination of ESBLPE in cattle farms, and to identify modifiable risk factors for possible intervention. More detailed molecular data are required in order to explore the role of horizontal gene transfer in the dissemination of the *bla*$_{ESBL}$ gene in this population and the risk of transfer to humans.

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Table 1. Farming types and prevalence of ESBLPE in Israeli farms.

Farm type	Farms (N)	Sections (N)	ESBLPE		
			Mean +/- SD	PR[3]	P-value
Dairy Cattle	17	66	2.6 +/- 3.2	0.611	0.03
Fattening Farm	7	16	2.5 +/- 3.3	0.576	
Pastoral Farming	6	13	0.4+/- 1.4	0.074	
Mixed Pasture and Feedlot[1]	5	12	1.8+/- 2.6	0.436	
Quarantine Station	2	4	4.3+/- 3.3	1.000	
Mixed Dairy Feedlot[2]	3	12	3.0+/- 3.4	0.865	

[1]- 6 sections of pasture and feedlot each; [2]- 9 dairy section and 3 feedlot; [3]-PR- prevalence ratio.

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Table 2. Descriptive statistics and bivariable analysis of variables related to ESBLPE carriage in Israeli cattle.

Covariate	Mean ± SD of Covariate\ Category	Mean ± SD of Prevalence of Bacteria	No. (%) of sections in Group	PR[2]	P-value
Numeric covariate					
Cattle cleanliness (%)	35.1 ± 34.5	2.4 ± 3.1	123 (100.0)[1]	1.010	<.001
Trough, N	3.1 ± 2.4	1.8 ± 2.5	111 (90.2)[1]	0.927	0.053
Categorical covariate					
Age (month)	<4m	5.3 ± 3.6	32 (26.0)	6.534	<.001
	5-10m	1.7 ± 2.6	29 (23.6)	2.136	
	11-24m	1.4 ± 1.9	36 (29.3)	1.545	
	>25m	0.9 ± 1.4	26 (21.1)	1.000	
Trough cleanliness	Dirty	2.6 ± 3.1	16 (13.0)	0.242	<.001
	Partially dirty	1.7 ± 2.3	82 (66.7)	0.226	
	Clean	3.9 ± 4.1	21 (17.1)	1.000	
	Missing		4 (3.3)		
Cooling System	Fan	2.5 ± 3.2	39 (31.7)	0.791	<.001
	Fan+ Nebulizers	1.2 ± 1.5	18 (14.6)	0.261	
	Nothing	2.6 ± 3.2	66 (53.7)	1.000	
Crowdedness (head/m^2)	<5m2	5.9 ± 3.5	24 (19.5)	8.656	<.001
	5-10m2	2.1 ± 2.9	23 (18.7)	3.532	
	11-20m2	1.6 ± 1.9	40 (32.5)	1.988	
	21-30m2	1.4 ± 2.3	20 (16.3)	1.689	
	>30m2	0.6 ± 1.4	16 (13.0)	1.000	
Manure Cleaning Method	Tractor	1.5 ± 2.1	38 (30.9)	0.282	<.001
	Automatic Shovel	1.7 ± 1.7	18 (14.6)	0.187	
	Slatted Floors	0.0 ± 0.0	6 (4.9)	0.000	
	Nothing	3.3 ± 3.6	46 (37.4)	1.000	
	Missing		15 (12.2)		
No. cattle heads per	<199	3.1 ± 3.3	18 (14.6)	1.000	<.001

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Covariate	Mean ± SD of Covariate\ Category	Mean ± SD of Prevalence of Bacteria	No. (%) of sections in Group	PR[2]	P-value
section	200-499	2.8 ± 3.1	46 (37.4)	0.710	
	500-799	2.1 ± 3.1	43 (35.0)	0.375	
	800-999	1.0 ± 2.2	16 (13.0)	0.340	
No. Veterinarian visits/week	0	1.2 ± 2.0	36 (29.3)	0.176	0.032
	1	2.4 ± 3.3	17 (13.8)	0.392	
	2	2.9 ± 3.2	60 (48.8)	0.527	
	3	3.4 ± 4.1	8 (6.5)	0.690	
	5	5.5 ± 4.9	2 (1.6)	1.000	
New cattle in farm in preceding month	Not new	2.3 ± 3.0	91 (74.0)	0.684	0.781
	Israel	2.3 ± 3.3	25 (20.3)	0.779	
	Overseas	3.1 ± 3.2	7 (5.7)	1.000	
Antimicrobial prophylaxis	No	1.3 ± 2.0	90 (73.2)	0.231	<.001
	Yes	5.2 ± 3.7	33 (26.8)	1.000	
Vaccination other than mandatory	Yes	2.3 ± 2.9	23 (18.7)	1.000	0.548
	No	2.4 ± 3.1	100 (81.3)	1.300	

[1]-the number of sections (in the continuous variable) where data were available; [2]-

PR-prevalence ratio.

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Table 3. Characteristics of calves' sections in dairy farms versus other sections in dairy farms and calves' sections in fattening farms.

Covariate		Calves' (<4 m) sections, dairy farms (N=17)	Dairy farms, other sections (N=58)	Calves' (<4 m) sections, fattening farms (N=11)
		Number of sections (%)		
Cooling System	Fan	9 (52.9)	17 (29.3)	5 (45.4)
	Fan+ Nebulizers	0	18 (31)	0
	None	8 (47.1)	23 (39.7)	6 (54.6)
Crowdedness (head/m^2)	<5m2	14 (82.3)	4 (6.9)	5 (45.4)
	5-10m2	2 (11.8)	11 (19)	4 (36.4)
	11-20m2	1 (5.9)	25 (43.1)	2 (18.2)
	21-30m2	0	16 (27.6)	0
	>30m2	0	2 (3.4)	0
Manure Cleaning Method	Tractor	0	30 (52.6)	4 (40)
	Automatic Shovel	0	18 (31.6)	0
	Slatted Floors	0	6 (10.5)	0
	None	14 (100)	3 (5.3)	6 (60)
	Missing	3	1	1
Antimicrobial prophylaxis	No	1 (5.9)	54 (93.1)	5 (45.4)
	Yes	16 (94.1)	4 (6.9)	6 (54.6)

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Figure 1. Geographical locations of farms according to farming type. Legend: green-dairy farms; red-pasture farms; yellow-mixed pasture and feedlot farms; grey- quarantine stations; black-mixed dairy and feedlot farms.

Figure 2. Age-dependent carriage of ESBL-producing Enterobacteriaceae (ESBLPE) according to farm type. The number of sections in each group is given inside the column. Sections in mixed farms were defined according to section.

Figure 3. Distribution of ESBL-producing *E. coli* REP-PCR types in the different sections of farm no. 12. REP-PCR types are titled F1 to F4.

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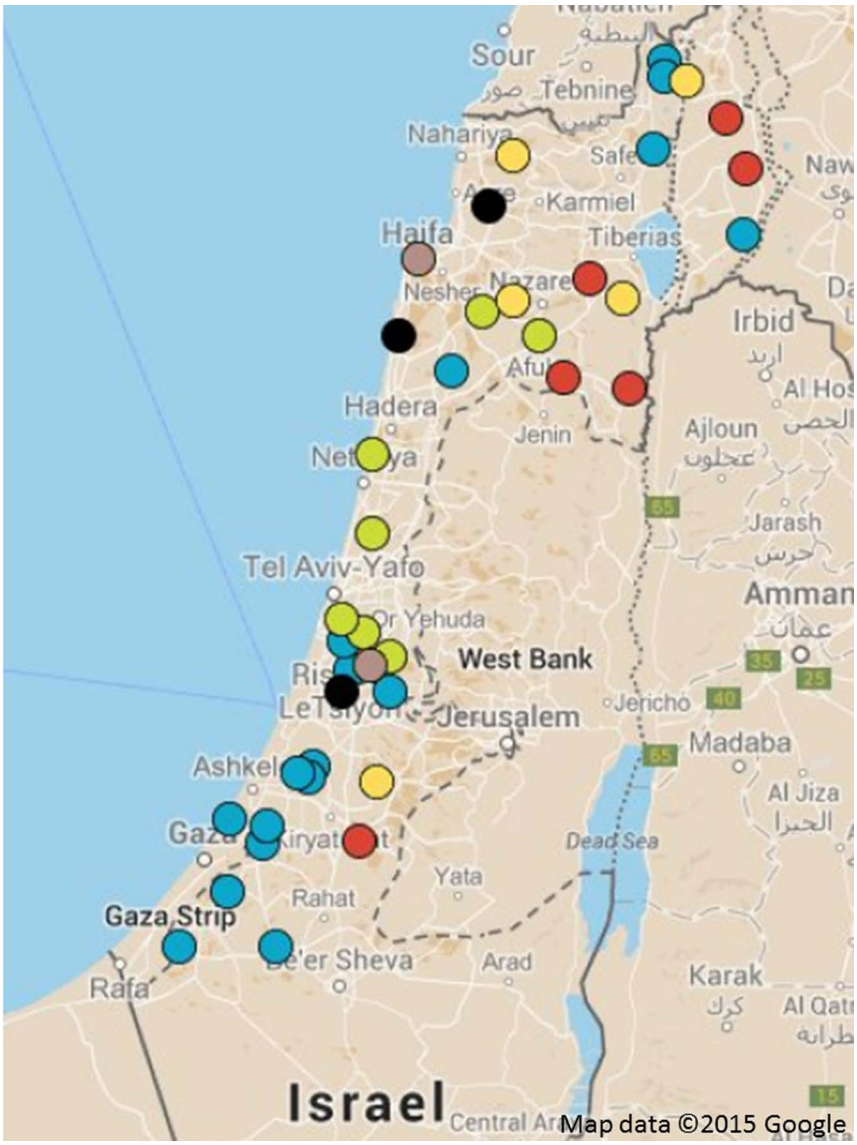

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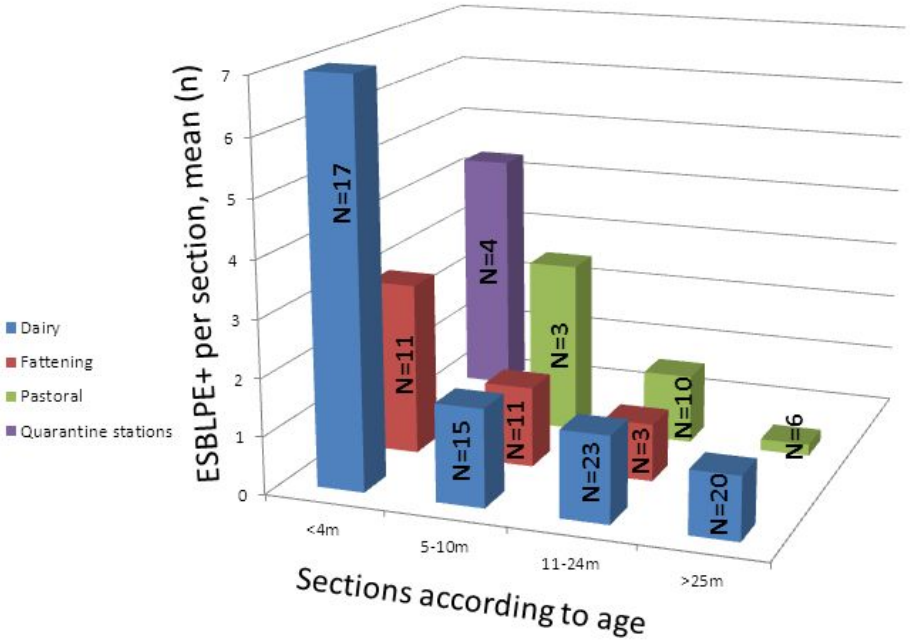

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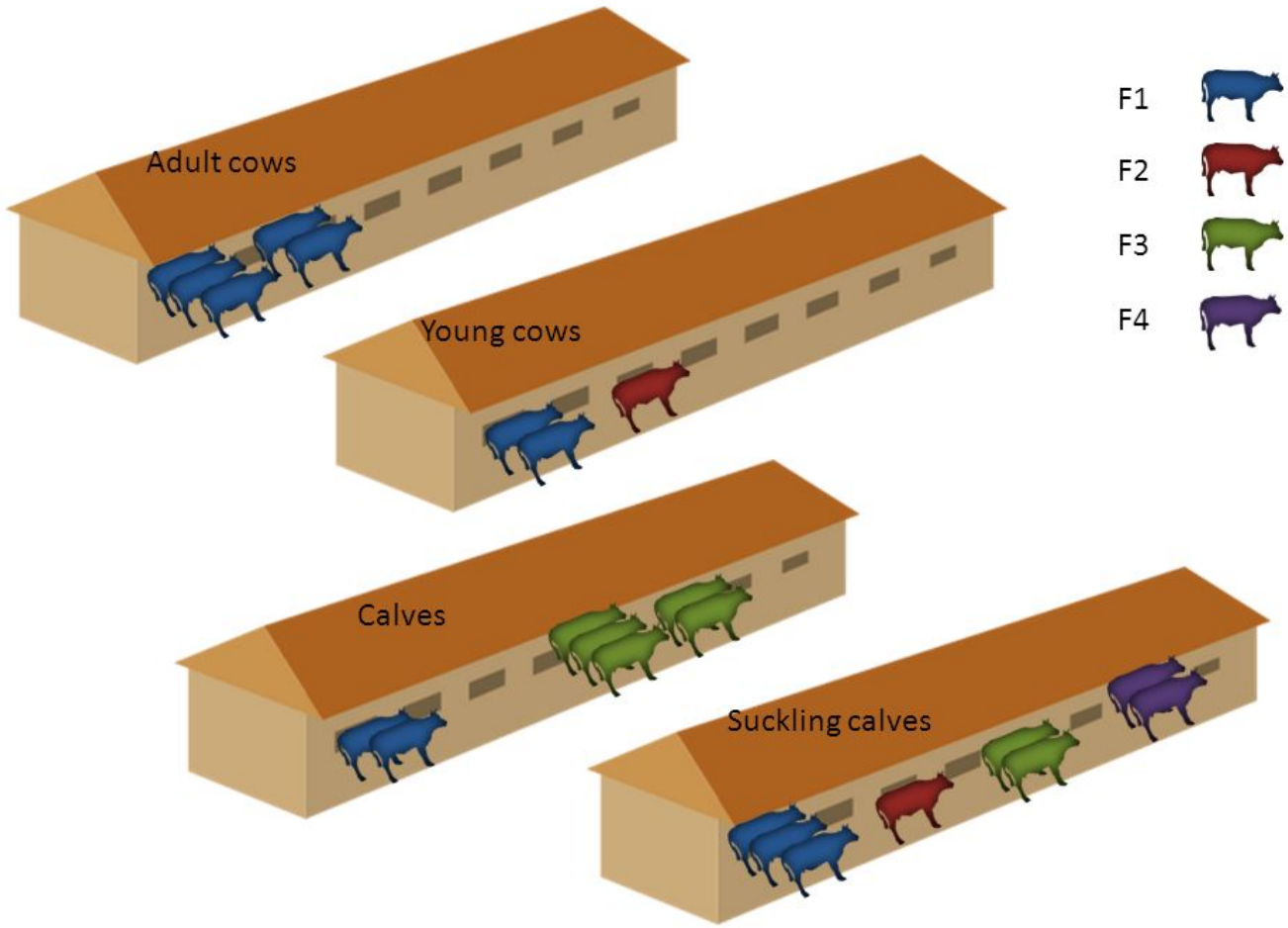

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