Investigating the Dominant *Escherichia coli* Strain in Lambs and Ewes Using Pyroprinting: A Novel Method for Strain Identification

CAL POLY

J. Nguyen, J. Vanderkelen, M. Black, C. Kitts

Abstract

Escherichia coli is a bacterium commonly found in the intestines and feces of animals. Due to the vast number of strains of *E. coli*, a specific host can often be identified by the dominant strain they carry in their intestines. This study looked at differences in dominant *E. coli* strains between ewes and their lambs. Although the lambs were sired by a single ram from a flock of ewes, the lambs were separated from the ewes after weaning. As a result, whether the dominant *E. coli* strains were stably maintained after early transfer events from the ewes to the lambs or if the lambs acquired their own dominant strain from the environment after separation from the ewes was able to be determined.

E. coli strains were isolated from fecal samples and categorized, using a new method referred to as pyroprinting. Pyroprinting involves simultaneously pyrosequencing the polymorphic, non-coding regions of all seven copies of *E. coli's* ribosomal RNA operons. The accumulation of mutations over time in these regions results in strain-specific patterns in the pyrogram output, referred to as a pyroprint. *E. coli* isolates purified from ewe or lamb fecal samples were classified into strains when pyroprints from both regions were matched based on Pearson correlation analysis using 99% as the lower threshold. Two isolates were collected and pyroprinted from each of 20 ewes and 25 lambs.

Pyroprint data from this study will be added to an expanding pyroprint library being developed at Cal Poly to aid in fecal source tracking studies. The amount of *E. coli* diversity present in sheep and the significance of heredity was explored. In addition, the discriminatory capability of pyroprinting one ITS region compared to the combination of two regions was looked into. It was found that the combination of the two regions provided significantly more discriminatory results than looking at either region independently. There were 48 lamb only isolates in 26 strains, 43 ewe only isolates in 17 strains, and only 3 isolates that belonged in one mixed strain. This data may indicate that the environment plays a larger role than heredity in the formation of a dominant *E. coli* strain in sheep and that lambs display a higher level of diversity than ewes.
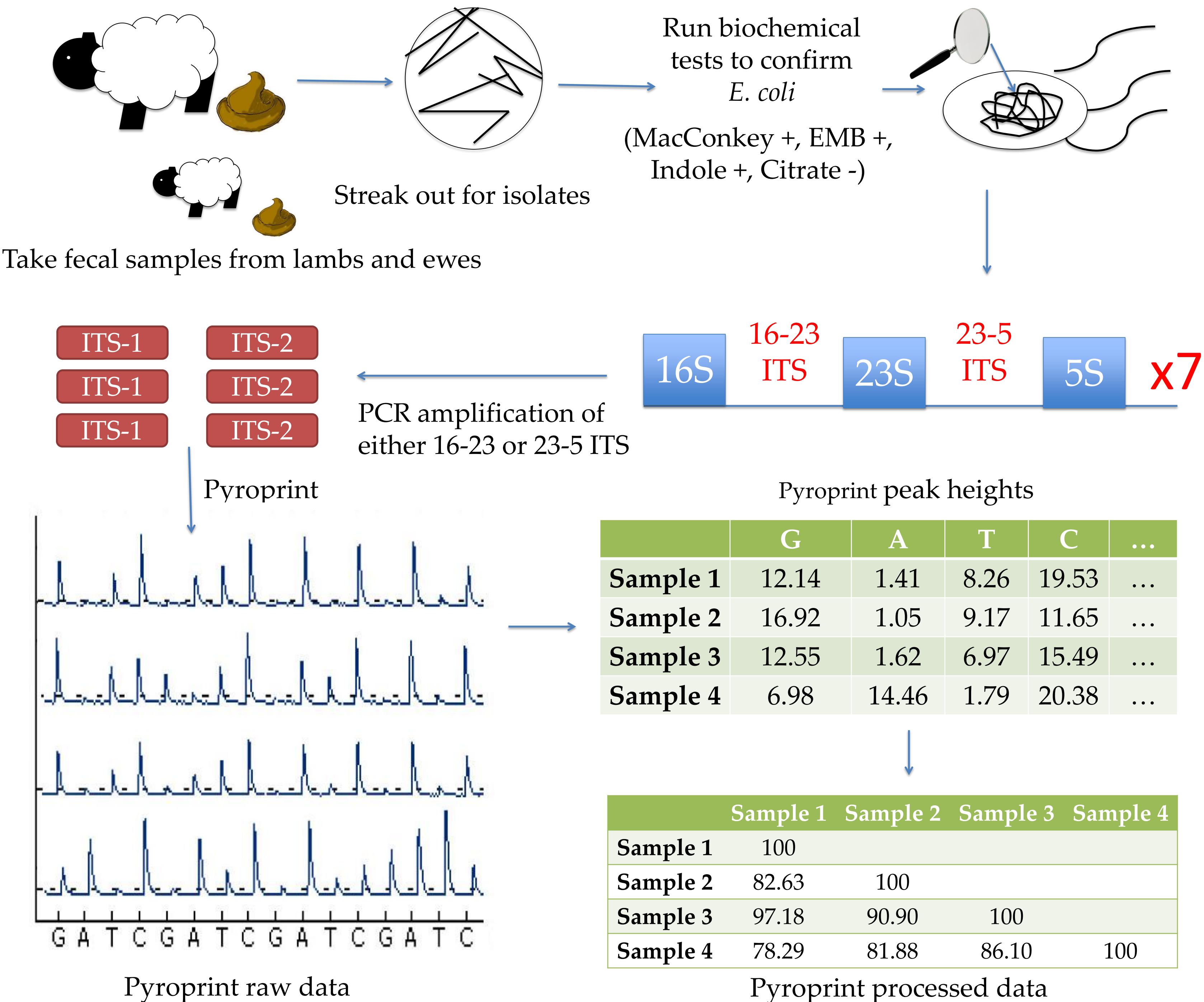
Introduction

Escherichia coli are motile Gram-negative bacilli that are present in large numbers in mammalian gastrointestinal tracts. *E. coli* is one of the first bacterial species to colonize the intestinal tract (3). Although they are usually commensal organisms, they are classified as opportunistic and certain strains can be a significant cause of disease. In addition to being a prominent cause of gastroenteritis in developing countries, *E. coli* is responsible for 80% of community acquired urinary tract infections and can also cause septicemia and neonatal meningitis (2).

Previous studies have shown that human infants can acquire *E. coli* both from their mother during vaginal childbirth and the environment (4). Around 33% of infants acquire the mother's dominant *E. coli* strain during childbirth (3). If the dominant strain from the mother was found in the infant, it was likely to become the dominant strain in the infant as well when the infant was sampled six months later (1). In developed countries human infants show a much lower dominant species turnover rate than in developing countries (3). This is mainly attributed to the cleanliness of the location. Infants born in a home setting are exposed to a greater variety of *E. coli* strains compared to those born in hospitals, and thus express a higher turnover rate.

In this study, pyroprinting was used to identify the dominant strains of *E. coli* in ewes and lambs. The research sought to discover if the dominant strain in lambs is maintained after early transfer events from their mothers or if an environmental strain took over and caused a turnover event. Another goal was to see if there is a greater amount of diversity of *E. coli* in lambs than in ewes since the ewes have had more time to acquire a dominant resident species. Lastly, the research served to look into whether or not it was beneficial to use the pyroprint data from both the 16-23S and the 23-5S ITS regions when clustering or if one region would suffice.

Hypotheses
1. **Similar to humans, lambs and ewes share 33% of their *E. coli*.**
2. **Lambs display a greater strain diversity than ewes.**
3. **Clustering using both the 23-5S and 16-23S ITS regions will be the most discriminatory.**

Method

Take fecal samples from lambs and ewes

Streak out for isolates

Run biochemical tests to confirm *E. coli*

(MacConkey +, EMB +, Indole +, Citrate -)

16S — 16-23 ITS — 23S — 23-5 ITS — 5S x7

PCR amplification of either 16-23 or 23-5 ITS

ITS-1 ITS-2
ITS-1 ITS-2
ITS-1 ITS-2
ITS-1 ITS-2

Pyroprint

Pyroprint peak heights

	G	A	T	C	...
Sample 1	12.14	1.41	8.26	19.53	…
Sample 2	16.92	1.05	9.17	11.65	…
Sample 3	12.55	1.62	6.97	15.49	…
Sample 4	6.98	14.46	1.79	20.38	…

	Sample 1	Sample 2	Sample 3	Sample 4
Sample 1	100			
Sample 2	82.63	100		
Sample 3	97.18	90.90	100	
Sample 4	78.29	81.88	86.10	100

G A T C G A T C G A T C G A T C

Pyroprint raw data

Pyroprint processed data

Results

Distribution of *E. coli* Strains into Clusters Using Different Loci Combinations
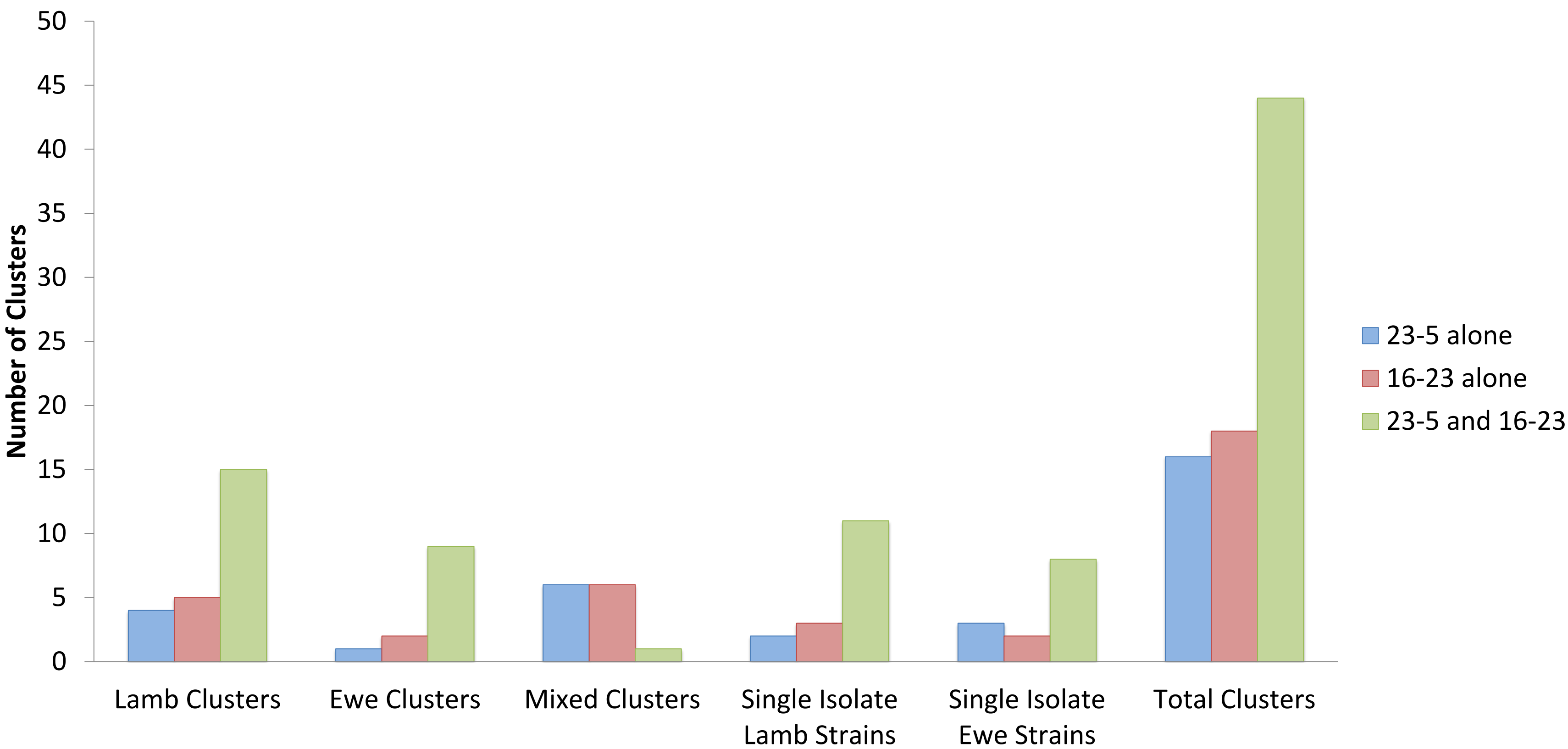

Figure 1: The number of clusters that were lamb only, ewe only, mixed, or single isolate categories. Different bars represent different loci combinations used in clustering.

Results

Distribution of *E. coli* Isolates into Categories Via Different Loci Combinations
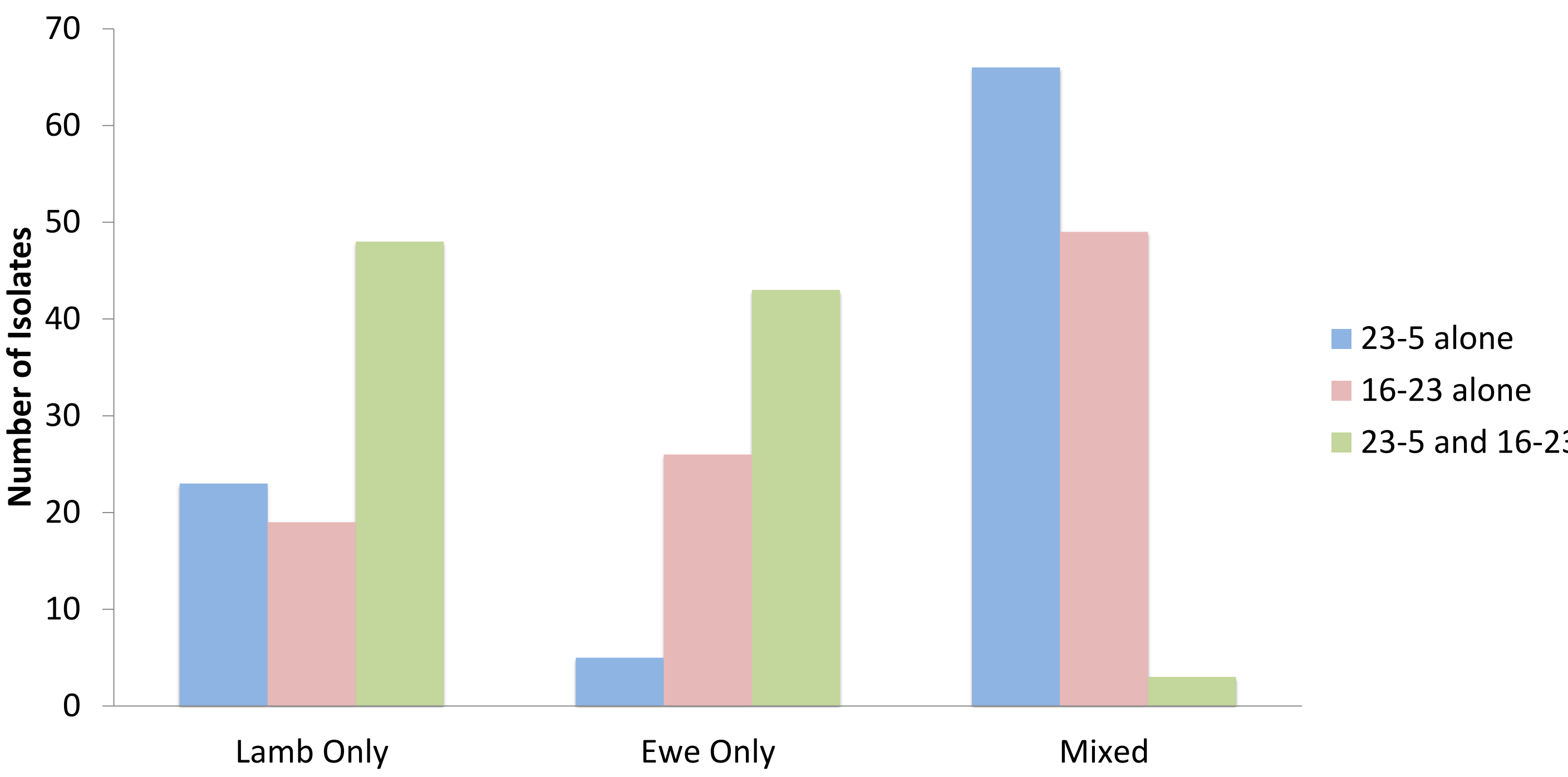

Figure 2: The number of isolates that fall into lamb only, ewe only, or mixed categories. Different bars represent different loci combinations used in clustering.

Conclusion

Ewes and lambs generally do not share *E. coli* strains
- Only 3 out of 94 isolates (roughly 3.2%) are shared

Lambs are more diverse than ewes
- There are 48 lamb only isolates in 26 strains compared to 43 ewe only isolates in 17 strains

These results may indicate that the environment plays a larger role than heredity in the formation of a dominant *E. coli* strain in sheep

Clustering using both loci is more discriminatory than just using one loci
- Total number of clusters jumps from 16/18 for 23-5S/16-23S to 44 when both loci are combined

Acknowledgements

References

1. Adlerberth, I., F. Jalil, B. Carlsson, L. Mellander, L. A. Hanson, P. Larsson, K. Khalil, and A.E. Wold, 1998. High turnover rate of Escherichia coli in the intestinal flora of infants in pakistan. *Epidemiology and Infection* 121:587-598.
2. Murray, P. R., K. S. Rosenthal, and M. A. Pfaller. 2009. In *Medical Microbiology*, 6th ed. W. Schmitt, and K. DeFrancesco, eds. Mosby/Elsevier, Philadelphia. p. 301-307.
3. Nowrouzian, F., B. Hesselmar, R. Saalman, I. Strannegard, N. Aberg, A. E. Wold, and I. Adlerberth, 2003. Escherichia coli in infants' intestinal microflora: colonization rate, strain turnover, and virulence gene carriage. *Pediatric Research* 54: 8-14.
4. Penders, J., C. Thijs, C. Vink, F. F. Stelma, B. Snijders, I. Kummeling, P. A van den Brandt, and E. E. Stobberingh, 2006. Factors influencing the composition of the intestinal microbiota in early infancy. *Pediatrics* 118: 511-521.