

# Indole negative STEC isolates

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# Background

## ■ *E. coli* confirmation according to ISO

ISO/TS13136 NOTE 5 Colony confirmation as *E. coli* can be achieved by using any commercial biochemical multi-assay **or** by assessing the production of indole. Confirmation of the serogroup can be achieved either by PCR or by agglutination with commercial antisera.

ISO/CD13136-1 NOTE 3 Colony confirmation as *E. coli* can be achieved by using any commercial or in-house validated biochemical multi-assay **or** by assessing the production of indole.

stx positive & indole negative -> presumable STEC / STEC not detected

- WFSR: stx positive & indole negative & API20E
- 5% (116) of all STEC isolates in WFSR-BioBank (2346) are indole negative
- Literature: 96% of *E. coli*'s are indole positive



[J. Bacteriol.](#) 2004 Nov; 186(21): 7460-7465.  
doi: [10.1128/JB.186.21.7460-7465.2004](#)

PMCID: PMC523188  
PMID: [15489459](#)

Molecular Basis of the Indole-Negative Reaction in *Shigella* Strains: Extensive Damages to the *tna* Operon by Insertion Sequences

[Ferdousi Rezwani](#),<sup>1</sup> [Ruifeng Lan](#),<sup>2</sup> and [Peter R. Reeves](#)<sup>1\*</sup>

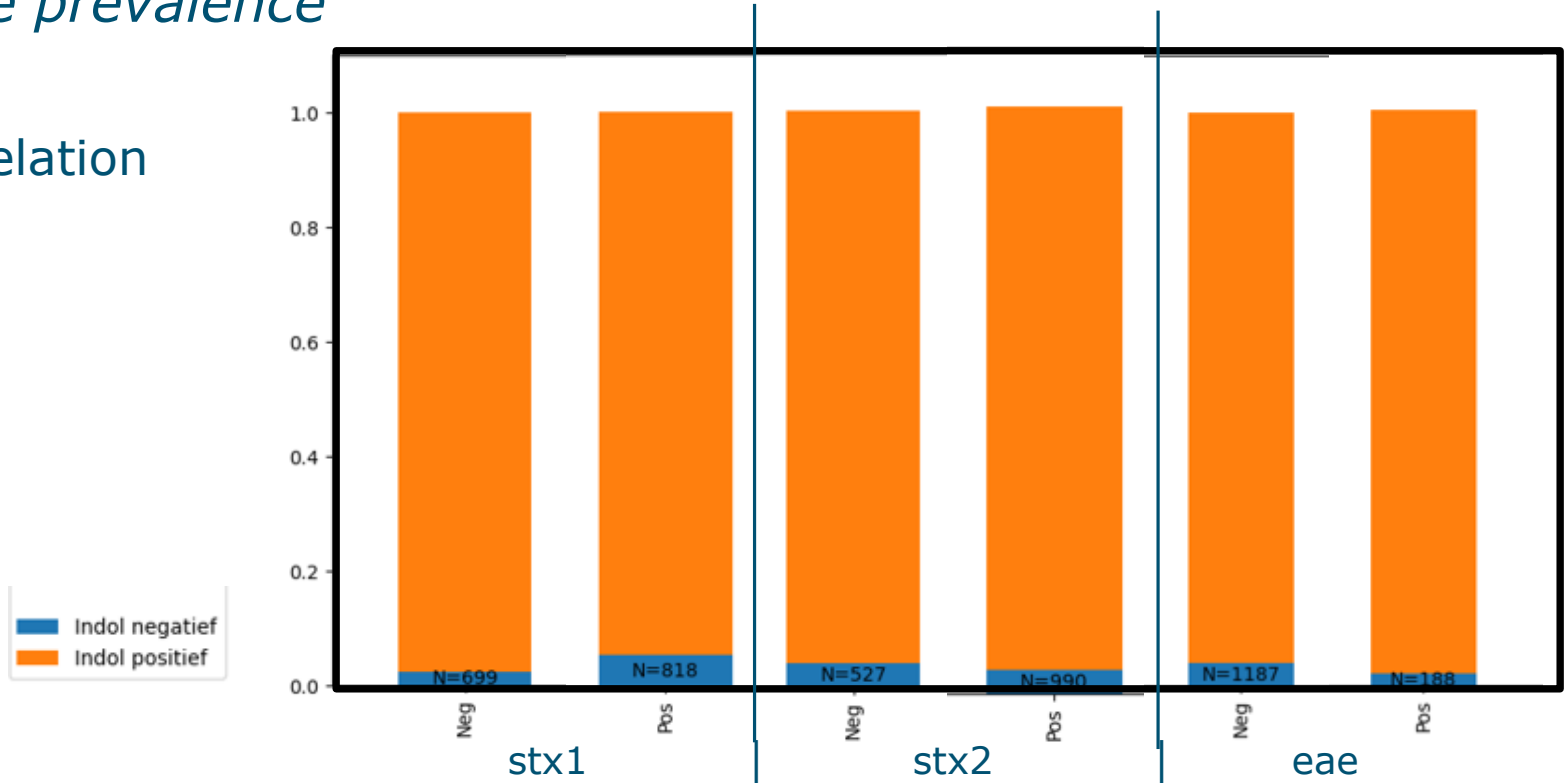
# Indole negative STEC isolates – data analysis

- Is there a correlation between indole negative STEC isolates and stx genes; product type or product group; animal species or serotypes?
- Dataset: WFSR BioBank STEC isolates: from 2017 to May 2023  
1483 STEC isolates in total  
61 isolates are indole negative (4%)

# Correlation indole negative STEC & stx/ea

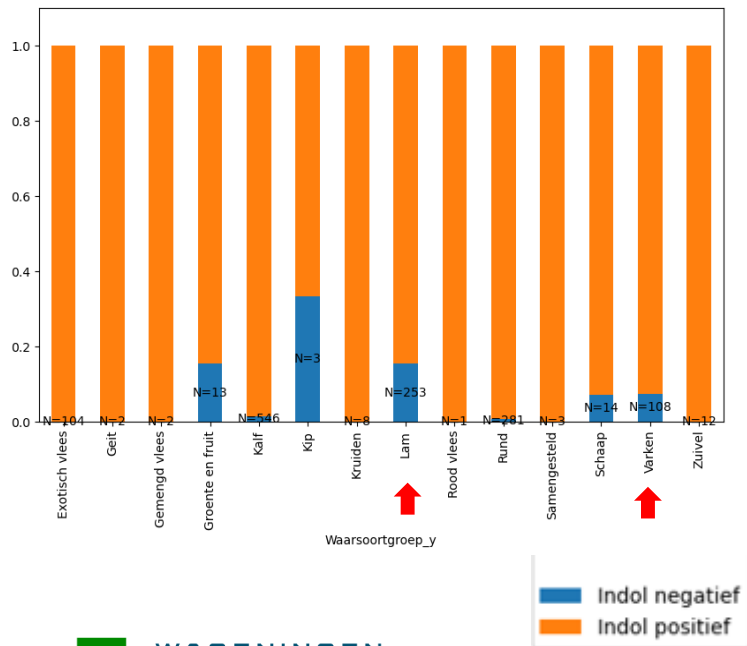
*Relative prevalence*

- No correlation



# Indole negative STEC & product species origin

## Relative prevalence



Species	% indol negative STEC isolates	#(total)
Lamb	15%	39(253)
Pig	6%	7(108)
Fruits/vegetables	8%	(1/13)
Sheep	7%	(1/14)
Chicken	33%	(1/3)
Calf	1%	(8/546)

# Indole negative STEC & species/product type

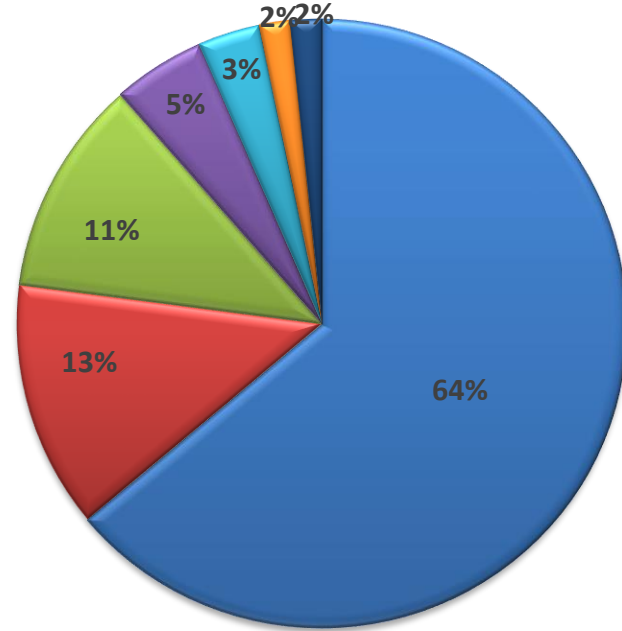
## *Relative prevalence*

- Indole negative & **species origin**
  - Relative high prevalence in: **lamb** (15%), **pig** (7%)
- Indole negative & **product type**
  - Relative high prevalence in:

<b>Product type</b>	<b>% indol negative STEC # (total)</b>
Racks	31% (12/39)
Chops	16% (18/113)
Meat	11% (9/82)
Meat preparations	5% (2/42)
<b>Manure</b>	6% (8/133)

# Indole negative STEC & species origin

64% (39 out of 61)  
indole negative STEC isolates  
originate from **lamb**

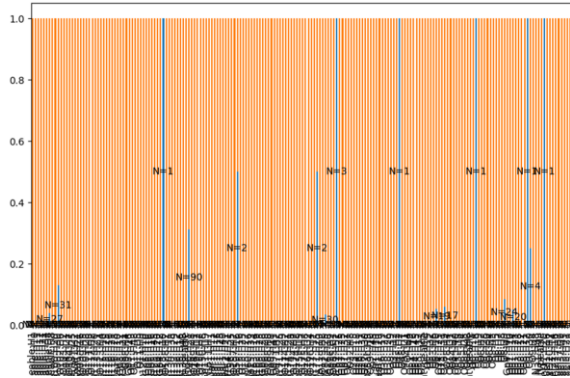


- Lamb
- Bovine
- Chicken

- Calf
- Fruits/vegetables
- Sheep
- Pig

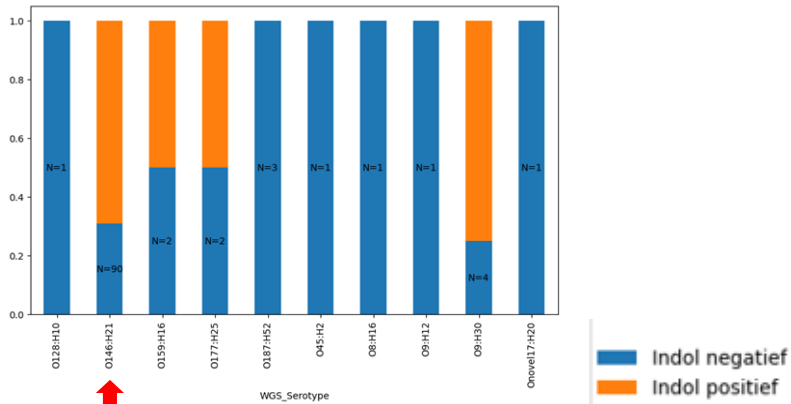
# Indole negative STEC & WGS serotype

## Relative prevalence



Serotype	% indol negative	STEC #(total)
<b>O146:H21</b>	<b>31%</b>	28(90)
O187:H52	100%	3(3)
O100:H30	13%	(4/31)
O08:H9	8%	(2/24)

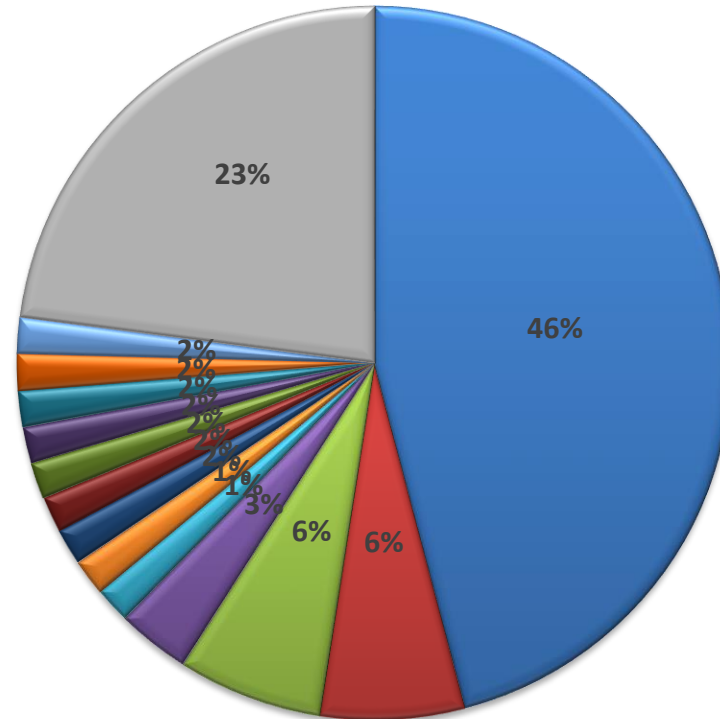
WGS serotypes filtered: > 0,2 negative





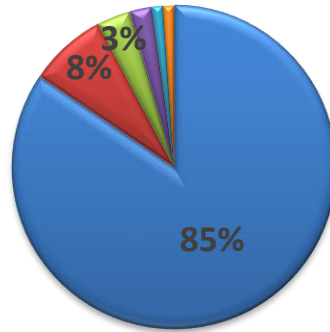
# Indole negative STEC & WGS serotype

46% (28 out of 61)  
indole negative STEC isolates has  
**O146:H21** WGS serotype

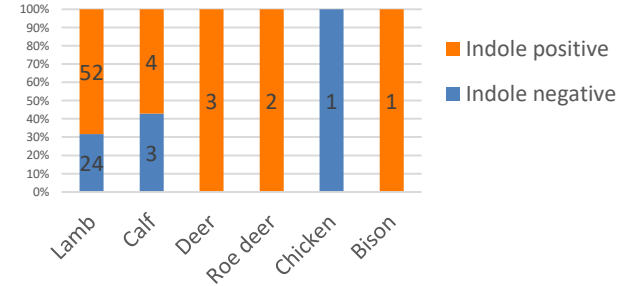


# Serotype O146:H21

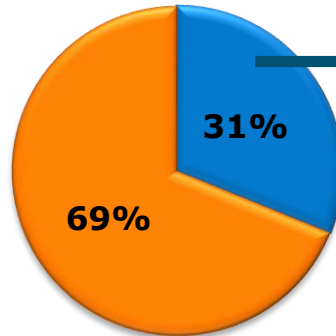
85% (76 out of 90)  
O146:H21 STEC isolates  
originate from Lamb



■ Lamb ■ Calf ■ Deer ■ Roe deer ■ Chicken ■ Bison



31% (28 out of 90)  
O146:H21 STEC isolates  
is indole negative



■ Indole negative ■ Indole positive

86% (24 out of 28) O146:H21  
indole negative STEC isolates  
originates from lamb meat.

From these 24 isolates:

23 - stx1 positive

16 - stx2 positive

0 - eae, aggR, aaiC positive

# O146:H21 in lamb

- 2021 Food monitoring (The Netherlands):

In total, 248 STEC isolates were identified, encompassing 83 different serotypes. Most predominant serotype found was O146:H21 (n=26), mainly in lamb meat.

- 2022 Food monitoring (The Netherlands):

In total, 103 STEC isolates were identified. The majority (40.8%) of the identified isolates originated from lamb meat (n=42). The most frequent detected serotype was O146:H21 (n=13 (n=12 lamb meat)).

- 2016 Surveillance program dairy goat and sheep farms

> J Appl Microbiol. 2023 Jun 1;134(6):lxad119. doi: 10.1093/jambio/lxad119.

## Virulence and antimicrobial resistance of Shiga toxin-producing Escherichia coli from dairy goat and sheep farms in The Netherlands

Angela H A M van Hoek<sup>1</sup>, Seungeun Lee<sup>2</sup>, Redmar R van den Berg<sup>3</sup>, Michel Rapallini<sup>2</sup>, Lennert van Overbeek<sup>2</sup>, Marieke Opsteegh<sup>1</sup>, Indra Bergval<sup>1</sup>, Ben Wit<sup>4</sup>, Coen van der Weijden<sup>4</sup>, Joke van der Giessen<sup>1</sup>, Menno van der Voort<sup>2</sup>

287 unique STEC isolates were obtained from fecal samples from dairy goat and sheep farms. Most predominant serotype found was O146:H21 (n = 65).

# Conclusion

- 64% from WFSR indole negative STEC isolates (2017-2023) originates from **lamb**
- 46% from WFSR indole negative STEC isolates (2017-2023) has the **O146:H21 serotype** (85% originated from lamb)
- It is advised to do additional confirmations for *E.coli* after an indole negative result of an isolated strain which is *stx* positive.  
Especially when the tested product is from lamb origin.

# Acknowledgements

- Denise van de Kamer (WFSR)

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Nature and Food Quality*

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