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Findings, conclusions & recommendations from two ring trials on the detection and isolation of Shiga (Vero) toxin -producing *E. coli* (STEC) from minced meat

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NRL-E.coli: organization of ringtrials for STEC detection

For quality assurance, the NRL *E. coli* has organized two ring trials in 2008 and 2009 on the detection and isolation of Shiga (Vero) toxin-producing *E. coli* (STEC) from minced meat samples.

In Germany, all kinds of STEC are regarded as potential human health hazards, independent of their serotype.

Methods used for detection of STEC from food in Germany

Methods used by food inspection laboratories for detection and isolation of STEC are based on officially recommended protocols (§64 LFGB).

These employ direct detection of Shiga (Vero) toxins (Stx) by Stx-ELISA followed by isolation using an Stx-Colony Immunoblot,

or stx-PCR for detection of *stx*-genes followed by colony DNA hybridization using *stx*-specific gene probes.

Ring trial participants

Fig 1: participant‘s locations

Food inspection laboratories in Germany and one in Switzerland.

23 laboratories participated in 2008,

26 laboratories participated in 2009

Governmental food inspection laboratories

University laboratories

Private laboratories

Sample Preparation

for each trial 10 kg of minced meat were produced at the BfR

The meat was checked for its microbiological status and for absence of STEC. Meat samples were frozen in aliquots of 25g in plastic tubes.

For the ringtrial, meat samples were encoded by numbers (1-9) and inoculated with defined quantities of STEC.

Meat samples were kept at 4°C and sent immediately by courier to the participants including the organizer's laboratory (NRL-E.coli)

STEC positive and negative samples

RT 2008 samples

RV 2008 sample	STEC	Stx-type[a]	average no. of STEC per 25g minced meat
1	O2:NM	Stx2-O118	135
2	-	-	-
3	O8:H19	Stx2	185
4	-	-	-
5	O113:H21	Stx2 & Stx2d	10
6	O8:H19	Stx2	1-5
7	-	-	-
8	O91:H14	Stx1	145
9	-	-	-

RT 2009 samples

RV 2009 sample	STEC	Stx-type[a]	average no. of STEC per 25g minced meat
1	O157:[H7]	Stx1 & Stx2	20
	O8:H19	Stx2	140
2	-	-	-
3	O145:[H28]	Stx2	20
4	O91:H14	Stx1	10
5	-	-	-
6	O2:NM	Stx2-O118	20
7	-	-	-
8	-	-	-
9	O103:H2	Stx1	10

For RT 2008 and RT 2009, each participant received five samples of 25g minced meat containing STEC and four samples without STEC together with a questionnaire asking about the conditions and methods used for STEC detection and isolation.

STEC isolates were sent from the participants to the NRL-E.coli for control.

Media used for STEC enrichment

Enrichment medium	RV2008	RV2009
m-TSB + novobiocin (20mg/L)	19 (82.6%)	24 (92.3%)
Brilliant Green Lactose Bile Broth	2 (8.6%)	2 (7.7%)
other	2 (8.6%)	0
Stx-Enhancer (mitomycin C)	13 (56.5)	17 (65.4)

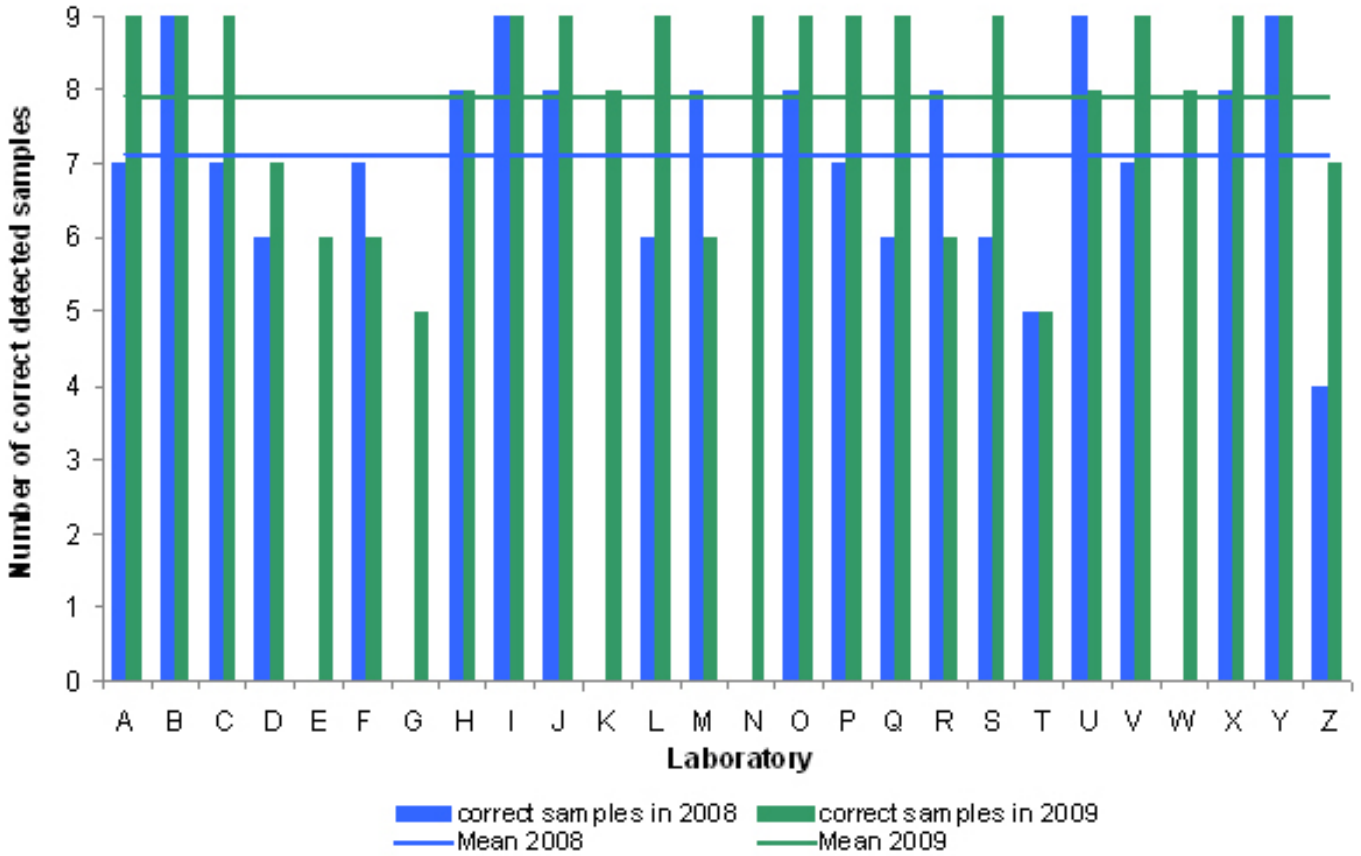
Methods for STEC detection

STEC detection method	RV2008	RV2009
Stx-ELISA (4 commerical tests)	11 (47.8%)	13 (50,0%)
stx-PCR	8 (34.8%)	8 (30.8%)
Stx-ELISA+*stx*-PCR	4 (17.4)	5 (19.2%)

Methods for STEC isolation

STEC isolation method	RV2008	RV2009
colony-immunoblot	14 (60.9%)	19 (73.1%)
colony-DNA hybridization	4 (17.4%)	4 (15.4%)
stx-PCR colony screening	3 (13.0%)	2 (7.7%)
cultural method only	2 (8.7%)	1 (3.8%)

Correct identification of samples



The average of correctly identified samples (STEC positive or negative) increased from 7 in RT2008 (blue) to 8 in RT2009 (green)

The number of laboratories identifying correctly all nine samples increased from four (17.4%) in RV2008 to 14 (53.8%) in RV2009.

interlaboratory variations between participants

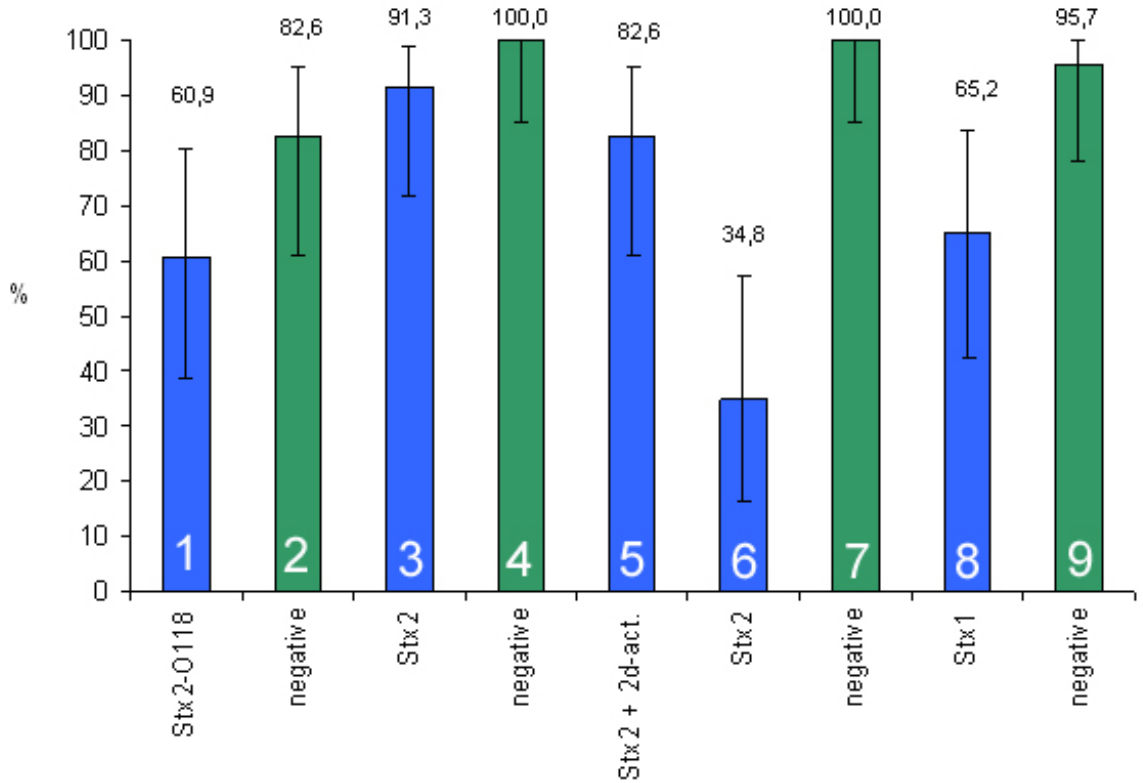
	samples	RV2008	RV2009
accordance (%)	positive (n=5)	69.4	80.9
	negative (n=4)	92.9	98.6
concordance (%)	positive (n=5)	55.1	66.5
	negative (n=4)	89.6	98.1
concordance odds ratio (COR)	positive (n=5)	1,26 (p=0.029)	1.22 (p<0.001)
	negative (n=4)	1.04 (p=0,3)	1.00 (p=1)

Detected by calculation of accordance, concordance and concordance odds ratios.

Accordance and concordance increased from RT2008 to RT2009 reflecting the lower number of false results in RV2009.

The value for concordance was lower than that of accordance indicating significant variations between the participants which was confirmed statistically.

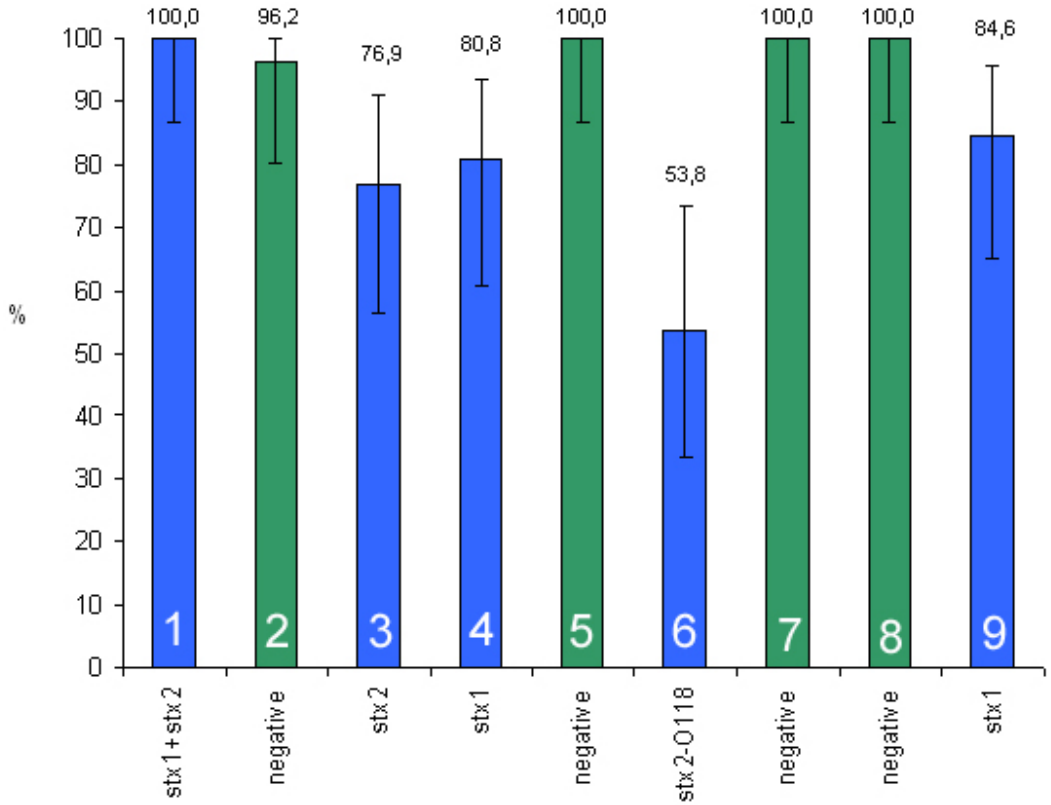
Detection sensitivity and specificity for the RT 2008



164 (79.2%) of 207 samples were correctly identified.

Differences in sensitivity may be caused by the amount of toxin produced in the sample (Stx-ELISA) or toxin (geno)type (Stx-ELISA / stx-PCR

Detection sensitivity and specificity for the RT 2009



206 (88,0%) of 234 samples were correctly identified.

Differences in sensitivity may be caused by the amount of toxin produced in the sample (Stx-ELISA) or toxin (geno)type (Stx-ELISA / stx-PCR) .

Comparison of STEC detection methods: Stx-ELISA & Stx-PCR

	STEC detection method	right (%)	false negative (%)	false positive (%)	total	p-value
RV2008	PCR	91 (84.3)	12 (11.1)	5 (4.6)	108	<0,01
	ELISA	73 (73.7)	26 (26.3)	0 (0.0)	99	
total		164	38	5	207	
RV2009	PCR	107 (91.4)	9 (7.7)	1 (0.9)	117	0,1
	ELISA	99 (84.6)	18 (15.4)	0 (0)	117	
total		206	27	1	234	

The sensitivity for Stx-ELISA was low (52.7%) in RT2008. This was due to the use of a non-suitable commercialized Stx-ELISA (Novitek) by 9 participants. The sensitivity for the Stx-ELISA increased to 72.2% in RV2009, only 4 participants still used this Stx-ELISA.

False-positive results were only obtained by use of stx-PCR. The number of false-positive results dropped from 5 (RT2008) to one (RT2009). There were no more statistically significant differences between Stx-ELISA and stx-PCR in RT2009 ($p=0.1$), in contrast to RT2008 ($p < 0.01$).

Detection and isolation of EHEC O157:H7

Sample 1 from RT2009 contained 2 STEC strains (O8:H19 & O157:H7)
The sample was detected as STEC-positive by all participants.

STEC O8:H19 was isolated by 25 (96.2%) participants, EHEC O157:H7 only by 2 participants (7.7%).

EHEC O157:H7 was added at lower numbers (20 cfu/25g meat) than STEC O8:H19 (140 cfu/25g meat).

This, and the non-employment of specific enrichment protocols for EHEC O157 (such as IMS, SMAC) may explain the low recovery rate for the EHEC O157 strain

Conclusions

An general improvement of the food inspection laboratories in Germany towards a better detection and isolation of STEC was observed by comparing results from ringtrials performed in 2008 and 2009.

As isolation methods for all types of STEC the colony immunoblot and the colony DNA hybridization are most promising.

Recommendations

The example of the “Novitek Stx-ELISA” shows that commercially obtainable tests which are not evaluated by independent sources should not be used for STEC detection as they may show deficiencies.

Users should not rely only on the declaration of the manufacturer.

The still growing number of Stx-subtypes requests that diagnostic labs evaluate their own test systems with a panel of Stx-reference strains to know which toxin types are detectable.

The low isolation rate for O157 from a sample containing two STEC strains shows that the ISO 16654 method should be used in parallel for an optimized isolation of EHEC O157.

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