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Genotyping *Mycoplasma hyopneumoniae* isolates based on multi-locus sequence typing, multiple-locus variable-number tandem repeat analysis and analysing gene *p146*

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ABSTRACT

Mycoplasma hyopneumoniae is a swine pathogen bacterium, causing significant economic losses worldwide. Epidemiological investigations based on molecular typing methods support the prevention and eradication strategies for the control of *M. hyopneumoniae,* through tracing the spreading of the pathogen. The present study describes the genotyping of 44 *M. hyopneumoniae* strains isolated from Hungarian, Czech and Slovakian porcine lung samples by multi-locus sequence typing (MLST), multiple-locus variable-number tandem repeat analysis (MLVA) and analysing gene *p146,* and the evaluation of the used methods. The resolution of the three-gene (*adk*, *rpoB*, *tpiA*) and the seven-gene (*efp*, *metG*, *pgiB*, *recA*, *adk*, *rpoB*, *tpiA*) based MLST systems was identical with 27 sequence types. MLVA utilising loci P97-RR1 and Locus1 extended with the serine repeat numbers of gene *p146* showed the highest resolution power among the studied methods differentiating 40 genotypes. The independent analysis of gene *p146* revealed 31 different types among the isolates.

High variability of *M. hyopneumoniae* strains was detected by the used typing methods. The results confirmed that utilization of the minimal MLST is suitable for phylogenetic analyses of *M. hyopneumoniae* strains. The MLVA method extended with the evaluation of serine repeat numbers of gene *p146* is adequate for the resolution of genetic relationships within MLST groups. Examination of the *p146* gene is suitable to complement both MLST and MLVA methods in order to refine closer genetic relationships.

1. Introduction

Mycoplasma hyopneumoniae is the etiologic agent of porcine enzootic pneumonia, a contagious respiratory disease, characterised by chronic cough, growth retardation, low mortality, but high morbidity. Culturing the organism is the gold standard for diagnostic purposes, but the isolation of *M. hyopneumoniae* is fastidious and time-consuming, thus it is not used for routine diagnosis (Maes et al., 2008). Therefore, genotyping methods without prior cultivation are very useful in epidemiological investigations. Among the genotyping methods, conventional multi-locus sequence typing (MLST) analysis of *M. hyopneumoniae* utilises seven housekeeping genes (*efp*, *metG*, *pgiB*, *recA*, *adk*, *rpoB*, *tpiA*) to estimate phylogenetic relationships (Mayor et al., 2008). This is a well-established and frequently used method, which provides data easily comparable between different laboratories through online databases. Multiple-locus variable-number tandem repeat analysis (MLVA) is an inexpensive and fast method for epidemiological investigations of *M. hyopneumoniae* (Vranckx et al., 2011; Charlebois et al., 2014), but inter-laboratory comparisons of the results are difficult, because the target regions are not standardised and the majority of the sequence data are publicly unavailable. Another typing method of *M. hyopneumoniae* applicable directly on clinical material is the analysis of gene *p146*. It is an adhesin-like protein encoding region containing a variable-number tandem repeat (VNTR) region, which can be used for genotyping either by VNTR analysis (Vranckx et al., 2011; dos Santos et al., 2015; Michiels et al., 2017) or by sequence analysis (Mayor et al., 2007; Savic et al., 2010).

The aims of the present study were to perform the genotyping of a *M. hyopneumoniae* strain collection based on the analyses of gene *p146,* MLST and MLVA data and to evaluate the used molecular typing

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Table 1
Background data of the *M. hyopneumoniae* strains including information about isolation, minimal MLST (multi-locus sequence typing) sequence and allele types, gene *p146* sequence types and number of tandem repeats of the MLVA (multiple-loci variable-number of tandem repeat analysis) loci and gene *p146* VNTR.

Data of isolation			MLST allele types					MLVA loci				
Sample ID	Country and herd of origin	Date of isolation	STs	*adk*	*rpoB*	*tpiA*	*p146*	P97-RR2	Locus2	P97-RR1	Locus1	*p146* (VNTR)
MycSu1	H, Hajduszoboszlo	2015	82	26	34	37	87	4	2	8	2	29
MycSu2	H, Hajduszoboszlo	2015	82	26	34	37	87	4	2	8	2	29
MycSu3	H, Nagyhegyes	2015	97	28	35	1*	88	3	2	11	3	18
MycSu4	H, Nagyhegyes	2015	98	29	36	38	89	3	2	7	6; 7; 9	18
MycSu5	H, Tata	2015	96	27	37	38	89	3	2	8	1	18
MycSu6	H, Tata	2015	96	27	37	38	90	3	3	8	1	17
MycSu7	H, Mezotur	2015	83	26	38	39	91	3	3	6; 11; 19	2	10
MycSu8	H, Mezotur	2015	82	26	34	37	92	4	2	5	2	32
MycSu9	H, Hajdunanas	2015	95	23*	39	40	93	4	3	8	10	13
MycSu10	H, Bekescsaba	2015	82	26	34	37	92	4	2	8	2	32
MycSu11	H, Bekescsaba	2015	82	26	34	37	92	4	2	8	2	32
MycSu12	H, Dombegyhaz	2015	80	23*	40	41	94	3	2	8	8	17
MycSu13	H, Rabaszentandras	2015	82	26	34	37	87	4	2	5	2	29
MycSu14	H, Csikostottos	2015	86	27	37	42	95	5	2	13	8	35
MycSu15	H, Bacsalmas	2015	89	30	41	7*	96	5	3	8	14	17
MycSu16	H, Bacsalmas	2015	89	30	41	7*	96	5	3	8	13	17
MycSu17	H, Palhalma	2016	83	26	38	39	97	3	2	11	2	17
MycSu18	H, Bacsalmas	2016	89	30	41	7*	96	5	3	9	11	17
MycSu19	H, Labod	2016	90	31	42	43	99	3; 4; 8	2	8; 10; 17	1	36
MycSu20	CZ, no data	2016	74	6*	43	44	98	4	2	6	5	15
MycSu33	H, Labod	2016	90	31	42	43	99	2; 3; 7	2	7; 8; 15	1	36
MycSu34	H, Oroshaza	2016	72	5*	37	42	100	5	3	12	7	33
MycSu37	H, Csemo	2016	91	32	44	38	101	2	2	11	1	28
MycSu39	H, Sellye	2016	85	27	15*	38	102	3	3	9	2	36
MycSu40	H, Felsobabad	2016	93	33	45	45	97	4	2	11; 12; 19	4	17
MycSu41	H, Mezotur	2016	89	30	41	7*	96	5	3	8	18	17
MycSu42	H, Mesterszallas	2016	82	26	34	37	103	3	2	7	2	31
MycSu43	SK, no data	2016	73	6*	37	38	104	5	2	9	15	42
MycSu44	SK, no data	2016	75	6*	46	46	105	2	2	6	9	17
MycSu45	H, Dobrokoz	2016	81	23*	47	41	106	3	2	6	9	27
MycSu46	H, Szentes	2016	79	19*	38	26*	107	3	1	9	2	26
MycSu47	H, Lovasbereny	2016	87	29	48	47	108	3	2	6	5	12
MycSu49	H, Felsobabad	2016	93	33	45	45	97	4	2	13	3	17
MycSu50	H, Fegyvernek	2016	78	19*	37	42	109	5	1	10	2	36
MycSu52	H, Baracska	2016	76	6*	49	1*	110	5	2	10	7	26
MycSu53	H, Ocsa	2016	82	26	34	37	111	5	2	8	17	31
MycSu70	H, Fabiansebestyen	2016	88	29	50	38	112	4	2	4; 9; 21	4	27
MycSu79	H, Papa	2016	92	33	18*	45	117	3	3	12	2	8
MycSu80	H, Nadudvar	2016	82	26	34	37	113	4	2	10	1	34
MycSu81	H, Nadudvar	2016	82	26	34	37	113	4	2	10	1	34
MycSu82	H, Nadudvar	2016	82	26	34	37	113	4	2	10	1	34
MycSu83	SK, no data	2016	77	6*	51	48	114	5	2	8	13	27
MycSu84	H, Osli	2016	84	26	52	49	115	3	2	7; 8; 15	2	19
MycSu85	H, Devavanya	2016	94	34	53	45	116	5	3	11	5	35
J strain			28*	5*	16*	16*	26*	5	4	7	9	18

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