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MOLECULAR EPIDEMIOLOGIC FEATURES OF LISTERIOSIS IN ANIMALS IN NEW YORK STATE

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Une étude rétrospective de l'association possible entre la forme clinique de listériose et les caractéristiques des souches isolées de foyers de listériose animale dans l'Etat de New York a été réalisée. Elle a évalué le rôle des allèles du gène de virulence dans la forme clinique de listériose. Les données de 40 foyers de cet Etat ont été réunies. Ces foyers avaient touché des moutons, des chèvres, des bovins et d'autres animaux, avec différentes manifestations cliniques. Les souches isolées ont été caractérisées génétiquement à l'aide de techniques moléculaires et l'association entre ces caractéristiques et la clinique, évaluée par analyse de régression logistique. Pour actA l'allèle 2 était associé de manière significative avec une augmentation du risque d'avortement. L'encéphalite était moins associée à l'allèle 2 d'actA qu'à l'allèle 1.

INTRODUCTION

There has been a growing attention to *Listeria monocytogenes* infection as the result of rapidly increasing number of outbreaks in farm animals with significant economic losses. Listeria, also a potential pathogen in man, is widely found in nature and was first described more than sixty years ago (Murray, 1926). Nevertheless, the epidemiology of this bacteria is highly complex and remained poorly understood. The organism, a Gram-positive, facultative anaerobic and facultative intracellular bacillus had been isolated in food related outbreaks in humans and animals.

Virtually all domestic and wild animals are susceptible to listeria infection (Ryser, 1991). Sheep appear to be more susceptible to the infection as several outbreaks had been reported around the world. There are three distinct clinical presentation of the disease that include: 1) septicemic form, which is common in young ruminants and characterized by being localized in the liver, spleen and other viscera (Ryser,1991); 2) a form in sheep and cattle which is characterized by metritis, placentitis, and abortion ; 3) and a meningoencephalitis form which is relatively common in sheep, goats and cattle (Ryser,1991).

The true incidence of listeriosis in both man and animals is largely unknown. The true source of transmission, and the epidemiologic structure of this disease are still unclear . However, most contagion and outbreaks of listeriosis reported contraction of disease through ingestion of contaminated food. Silage is often documented as the source of infection in farm animal outbreaks (Gray, 1960; Gitter et al., 1986). *L.monocytogenes* isolates have been characterized and classified into different groups using a variety of methods. These methods include serotyping, electrophoretic typing (Piffaretti et al., 1989), pulsed-field fingerprinting (Brosch et al., 1994) and ribotyping (Graves et al., 1994). Recently, Wiedmann et.al (1996) had defined three genotypic lineages of *L.monocytogenes* based on ribotypes and allelic PCR analysis of virulence gene *hly, inlA* and *actA*. There are currently fifty *L.monocytogenes* ribotypes which are grouped into eleven ribotype subsets by ribotyping. Allelic analysis (PCR-restriction fragment length polymorphism) of the virulence genes revealed eight *hly,* eleven *inlA* and two *actA*. Combination of these virulence genes alleles and ribotype pattern separate *L.monocytogenes* into three different lineages. Information and knowledge of the significance of these different lineages with regard to their role in overt animal disease is limited. There had been reports on the difference in the incidence of diseases in human by different genetic types of L. *monocytogenes*. There is evidence of differences in pathogenic potential among the three lineages. Lineage I is mainly implicated in epidemic outbreaks of human listeriosis while all three lineages had been isolated from cases in animals. Lineage III have not been linked with any human listeriosis cases.

We carried out a retrospective study investigating the possible association between the listeriosis clinical form and molecular characteristics of the isolates in outbreaks of listeriosis in animals of New York State and evaluating the role of specific virulence gene alleles in determining the type of listeria form .

MATERIALS AND METHODS

All confirmed cases of listeriosis referred to Cornell Diagnostic Laboratory(DL) from March1986 to Jun 1996 by veterinarians around New York State were included in this study. Details of each incidence of listeriosis abortion, encephalitis and septicaemia were collected and recorded in a systematic manner to serve as database. There were a total of 74 cases of listeriosis in sheep, goats, cows and other animals (eg horse, deer, gorilla). Each case was confirmed by i) histopathological examination of specific lesions ii) standard bacteriological culture. iii) polymerase chain reaction-based assay to determine its virulence genes and iv) ribotyping to determine the specific ribotype fingerprints. The specific ribotype and gene alleles thus determine the lineages. The available data from each case includes the listeria form manifested, species of animal and the

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exclusive lineages of *Listeria monocytogenes.*
Statistical analysis was carried out using BMDP statistical software (Dixon,1992). Chi-square analysis for categorical variables were carried out to measure the simple association between the hypothetical risk factor (ACTA, INLA, HLY , LINEAGES) and the listeric form. Then stepwise logistic regression was used to evaluate the effect of each risk factor for the dichotomous outcome while controlling for the others. The significance of each variable were considered at $\alpha = 0.1$. Variables were entered in the model if they explained a significant portion of the variation ($p<0.1$) in the likelihood ratio test. On the completion of the final model, the odds ratio and their 90% confidence intervals(CI) were calculated to estimate the strength of association.

RESULTS

There were a total of 40 cases of encephalitis, 18 cases of abortion, 12 cases of septicaemia and 4 cases of other forms of listeriosis observed. The details of the cases are presented in table I.

Table I
Distribution of 74 cases of listeriosis with specific listeria form experienced experienced from March 1993 to Jun 1996

	Sheep	Goat	Cow	Others
Encephalitis	11	11	16	2
Abortion	0	0	17	1
Septicaemia	2	0	6	5
Others	0	0	1	1

The results for the multivariate analysis between the risk factors and their expected outcome were shown in table II. It was found that allele 2 for *actA* to be significantly associated with increased risk of abortion. It was approximately 3-times more likely to isolate virulence gene allele 2 *actA* from aborted animals in comparison to virulence gene allele 1 *actA* when controlling for the source of the sample. In the case of encephalitis it was less likely to isolate allele 2 in comparison to allele 1 of *actA*.

Table II
Odds ratio and 90% confidence interval for the association between clinical form abortion and *inlA*, *hly*, *actA* and lineages

Factor	Regression Coefficient	Standard error	Odds Ratio	90% Confidence Interval
abortion				
actA 1	0		1.0	
2	0.96	0.695	2.62	0.8, 8.2
encephaliti s				
actA 1	0		1	
2	-2.41	1..05	0.09	0.01, 0.5

DISCUSSION

Up to present time, there have been limited work done in molecular epidemiology of listeriosis either in human or animals; literatures were scanty and to the investigators knowledge, little attempt was made to relate disease manifestations to the organism's genetic characteristics. This study identified the associatin allele 2 of *actA* as a potential with thris risk of abortion in listeriosis. The authors were quite aware of limitations of this study as the samples represented were only from selected cases, hence there is weak external validity and therefore little inferences could be make to the population other than the outbreak cases. Causality is beyond the presented paper and the authors did not wish to infer so, however the conclusion from the analysis in this study could be considered in future studies. With more efficient and sophisticated technology along advance molecular engineering, this could be the key to a better disease control and more refined diagnostic tools .

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