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Research paper

MHC haplotype and susceptibility to experimental infections (*Salmonella* Enteritidis, *Pasteurella multocida* or *Ascaridia galli*) in a commercial and an indigenous chicken breed

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ABSTRACT

In three independent experimental infection studies, the susceptibility and course of infection of three pathogens considered of importance in most poultry production systems, *Ascaridia galli*, *Salmonella* Enteritidis and *Pasteurella multocida* were compared in two chicken breeds, the indigenous Vietnamese Ri and the commercial Luong Phuong. Furthermore, the association of the Major Histocompatibility Complex (MHC) with disease-related parameters was evaluated, using alleles of the LEI0258 microsatellite as markers for MHC haplotypes.

The Ri chickens were found to be more resistant to *A. galli* and *S.* Enteritidis than commercial Luong Phuong chickens. In contrast, the Ri chickens were more susceptible to *P. multocida*, although production parameters were more affected in the Luong Phuong chickens. Furthermore, it was shown that the individual variations observed in response to the infections were influenced by the MHC. Using marker alleles of the microsatellite LEI0258, which is located within the MHC region, several MHC haplotypes were identified as being associated with infection intensity of *A. galli*. An association of the MHC with the specific antibody response to *S.* Enteritidis was also found where four MHC haplotypes were shown to be associated with high specific antibody response. Finally, one MHC haplotype was identified as being associated with pathological lesions and mortality in the *P. multocida* experiment. Although not statistically significant, our analysis suggested that this haplotype might be associated with resistance. These results demonstrate the presence of local genetic resources in Vietnamese chickens, which could be utilized in breeding programmes aiming at improving disease resistance.

1. Introduction

Indigenous breeds constitute the majority of the poultry production in developing countries and these breeds are usually small-sized with a low productivity. An often used strategy to increase productivity in livestock, including poultry, is therefore to introduce commercial

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exotic breeds/crosses and in many developing countries such breeds are now being imported, as well as produced locally in increasing numbers (Shand, 1997; Mendelsohn, 2003; Rege and Gibson, 2003; Anderson, 2003; Delany, 2003). However, the strategy of replacement or cross-breeding of indigenous breeds with exotic commercial hybrids, as well as the general spread of industrial livestock production, constitutes an increasing risk of losing local genetic traits, such as disease resistance, before they are described and studied (Shand, 1997; Anderson, 2003; Delany, 2003). An alternative strategy to increase productivity is to utilize already existing traits to breed for disease resistance (Lamont, 1989). Selection for genotypes associated with disease resistance can thus be a useful addition to disease control programmes and particularly one gene complex, the Major Histocompatibility Complex (MHC; in chickens also called the B-complex), has been investigated thoroughly for its role in disease resistance (Lamont, 1998). In chickens, the MHC has been shown to be involved in the genetic control of resistance to several viruses, including Marek's disease, Rous sarcoma virus and avian leucosis (Plachy et al., 1992, 2003; Yoo and Sheldon, 1992; Rothschild et al., 2000), as well as to various bacteria, such as *Pasteurella multocida*, *Salmonella* Enteritidis and *Staphylococcus aureus* (Lamont et al., 1987; Cotter et al., 1998; Liu et al., 2002). Furthermore, the MHC has been shown to be associated with resistance to coccidia (Clare et al., 1985; Lillehoj et al., 1989; Caron et al., 1997) and recently we reported the finding of an association between MHC and infection intensity of different helminth species in naturally infected chickens, including *Ascaridia galli*, *Heterakis beramporia* and *Tetrameres mothedai* (Schou et al., 2007).

In Vietnam, the total population of chickens in 2005 was estimated to approximately 154 million (FAOSTAT, 2007) of which indigenous breeds roughly constitute 75% (Tran, 2002). In the northern part of Vietnam, the indigenous breed, Ri, is the most widespread breed, especially among smallholder farmers. However, a commercial cross (Luong Phuong) of Chinese origin is now being produced at governmental breeding farms and sold as day-old chicks to farmers. Due to favourable prices and high productivity, the Luong Phuong breed has gained a significant share of the poultry production in recent years and the risk of losing genetic resources associated with disease resistance is therefore very tangible.

In the present study, Ri and Luong Phuong chickens were experimentally infected with three pathogens considered of importance in most production systems, *P. multocida*, *S.* Enteritidis and *A. galli*. *P. multocida* is the causative agent of fowl cholera which occurs in most countries, including Vietnam (Gunawardana et al., 2000; Glisson et al., 2003). In its acute stage, fowl cholera is associated with high morbidity and mortality and in chronic infections with lesions in the respiratory tract, including pneumonia (Glisson et al., 2003). *S.* Enteritidis is primarily of importance as a zoonotic agent, and although it can cause an increase in mortality of young chickens, it generally produces little systemic disease in adult chickens, in which it is able to colonize the alimentary and reproductive tract and thus enter the human food chain (Barrow et al., 2004). *A. galli* is one of the most frequently occurring parasitic nematodes in poultry (Ikeme, 1971a) and is widely recognized as significantly important due to infection-associated loss of weight, decreased egg production, anaemia, diarrhoea and mortality (Permin and Hansen, 1998). The objective of our study was to evaluate local genetic resources associated with disease resistance by comparing the susceptibility and the course of infection of these pathogens in the Ri and the Luong Phuong breeds. Moreover, we wanted to test for association of disease-related parameters with MHC haplotypes, using the LEI0258 microsatellite as a marker. This polymorphic locus is situated within the MHC region on chromosome 16 in a non-coding region between the B-F/B-L locus and the B-G locus (Fulton et al., 2006) and has previously been used as an MHC marker (Fulton et al., 2006; Schou et al., 2007).

2. Material and methods

2.1. Experimental animals and housing conditions

Three experimental infection studies were performed at the National Institute of Veterinary Research (NIVR) in Hanoi, Vietnam, using chickens of two breeds: Ri and Luong Phuong. In each of the three experiments, 100 chickens of each breed were inoculated with *A. galli* (Exp. 1), *S.* Enteritidis (Exp. 2) or *P. Multocida* (Exp. 3), respectively. Furthermore, 25 chickens of each breed were used as uninfected control animals in each experiment. All animals were purchased from a governmental breeding station, the Thuy Phuong Poultry Research Centre, and kept here until 1 week prior to inoculation at which time they were moved to the experimental facilities at NIVR.

In all three experiments, each group of chickens was housed in a separate room with concrete floor and sawdust bedding. Standard chicken feed and water were given *ad libitum*. In Exp. 1 and 2 the chickens were treated prophylactically against coccidiosis with Cocci-stop (clopidol). Until the age of 16 weeks, the chickens in Exp. 3 were kept at the Thuy Phuong Poultry Research Center where Cocci-stop was used routinely. All animals were vaccinated against Gumboro and Newcastle disease. The health status of the chickens was evaluated prior to the onset of the experiments and obvious weak birds were consequently excluded.

All work on experimental animals was carried out according to the International Guiding Principles for Biomedical Research Involving Animals as issued by the Council for the International Organizations of Medical Sciences. The experimental work on chickens in Vietnam was approved by the Ministry of Agricultural and Rural Development. The use of mice as an enrichment system for *P. multocida* was carried out with the approval of the Danish National Animal Ethics Committee.

2.2. Pathogens and inoculation

In Exp. 1, 2-week-old chickens were inoculated with embryonated *A. galli* eggs as follows: adult worms were collected from naturally infected hens in a layer flock. The female worms were dissected and the eggs embryonated in