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Haemolytic Complement Activity and Humoral Immune Responses to Sheep Red Blood Cells in Indigenous Chickens and in Eight German Dahlem Red Chicken Lines with Different Combinations of Major Genes (dwarf, naked neck and frizzled) of Tropical Interest

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ABSTRACT

A total of 376 chickens from different ecotypes were immunized with the non-pathogenic multi-determinant antigen sheep red blood cells (SRBC). The ecotypes included indigenous chickens from various locations in Tanzania (n = 102), India (n = 86) and Bolivia (n = 89). In addition, eight German Dahlem Red (GDR) chicken lines with different major genes (dwarf, naked neck and frizzled) of tropical interest were also immunized with SRBC. Immune competence of the breeds was assessed by measuring complement haemolytic activity, both from the classical calcium-dependent complement pathway (CPW) and alternative calcium-independent complement pathway (APW), alongside IgTotal, IgG and IgM antibody responses to SRBC at 7 days post immunization. Large variations in complement activity and antibody responses to SRBC were observed within and between the indigenous breeds. Many indigenous chickens, especially from Bolivia, showed decreased complement activity (APW) following immunization with SRBC. Breeds from India showed the highest CPW activity and humoral (especially IgM) responses to SRBC, suggesting high immune competence. In contrast, Bolivian chickens were characterized by low CPW activity, low APW activity and low antibody levels to SRBC suggesting an overall low immune competence. In the GDR chickens, characterized by high CPW activity and high IgG antibody responses to SRBC, the major genes for naked neck, frizzling and dwarfism had no significant effect on the antibody responses and complement activity to SRBC.

Keywords: complement, chicken, indigenous, Bolivia, India, Tanzania, sheep red blood cells, German Dahlem Red, naked neck, frizzled, dwarf

Abbreviations: APW, alternative (calcium-independent) complement pathway; CPW, classical (calcium-dependent) complement pathway; dw^-, dwarf gene; Ff^-, frizzling gene; GDR, German Dahlem Red; SRBC, sheep red blood cells; MER, mercaptoethanol-resistant antibodies; MES, mercaptoethanol-sensitive antibodies, MHC, major histocompatibility complex; $Nana^-$, heterozygous naked neck gene; PI, post immunization; SRBC, sheep red blood cells

INTRODUCTION

Immune parameters for assessing general immune competence in indigenous chickens should be chosen in such a way that the retained data rapidly yield a maximum of information to differentiate between disease-susceptible and disease-resistant chickens (Pandey *et al.*, 1992). Information regarding the immune status of native chickens (Kundu *et al.*, 1999) may be obtained reliably and quickly by immunizing birds with sheep red blood cells (SRBC) to test specific humoral immunity, and measuring complement activity as an innate immune response to SRBC (Haunshi *et al.*, 2002). Assessing haemolytic complement activity of the classical (CPW) and alternative (APW) pathways in combination with measuring antibody responses should also provide information on relationships between an important component of the innate immune system (APW) and innate immunity related to specific immunity (CPW), respectively.

SRBC is a T-cell-dependent multideterminant antigen. The use of antibody responses to SRBC as a multitrait selection criterion (Kean *et al.*, 1994a) is a well-studied but time-consuming concept in avian immunology that reveals various aspects of immune responses and their genetic basis. Heredity of antibody responsiveness to SRBC was estimated at 0.18 (Bovenhuis *et al.*, 2002). Immune response genes of the class IV (BG) region of the chicken major histocompatibility complex (MHC), influence primary antibody responses to SRBC, either by one dominant gene or through a gene complex, but there are also non-MHC influences (Dunnington *et al.*, 1996; Karaca *et al.*, 1999). For instance, the chicken MHC B21 haplotype is often associated with enhanced antibody responses and the B14 haplotype with low responses to SRBC (Martin *et al.*, 1990; Dunnington *et al.*, 1992; Pinard *et al.*, 1993; Kean *et al.*, 1994b; Gehad *et al.*, 1999). Effects of 'non-MHC genes' are revealed by the effects of route of administration (Kreukniet *et al.*, 1992; Boa-Amponsem *et al.*, 2001), dose (Kreukniet *et al.*, 1990) and time post immunization (Nelson *et al.*, 1995; Yang *et al.*, 1999; Boa-Amponsem *et al.*, 2000) on antibody responses to SRBC. Chickens selected for high antibody responses to SRBC have larger B-cell compartments (including larger germinal centres) in the spleen (Kreukniet *et al.*, 1996), higher levels of $CD4^+$ cells and lower levels of $CD8^+$ and TCR-1^+ cells than birds selected for low antibody responses (Parmentier *et al.*, 1996). Selection on the basis of antibody responses to SRBC revealed no consistent effects on cell-mediated immunity or macrophage activity (Pinard-Van der Laan, 2002). Positive correlations have been described between antibody responses to SRBC and enhanced responsiveness to *Escherichia coli* vaccine (Heller *et al.*, 1992), viral vaccines (Parmentier *et al.*, 1996) and protozoan infections (Gross *et al.*, 1980; Parmentier *et al.*, 2001). Finally, positive relations between selection for specific antibodies to SRBC and parameters of innate immunity (complement levels (Parmentier *et al.*, 2002) and natural antibodies (Parmentier *et al.*, 2004)) have been established. Although negative correlations were described between antibody responses to SRBC and responses to bacterial infections (Gross *et al.*, 1980) and body weight in selected chicken lines (Mashaly *et al.*, 2000), typing and selecting for antibody and complement responses to SRBC may improve the broad immune response of chickens.

Little information is available on the immune characteristics of indigenous (village) chickens. It is reasonable to assume that indigenous poultry breeds (ecotypes) have developed natural resistance to diseases through generations of exposure and natural selection. Characterization and evaluation of immune parameters in various ecotypes can offer knowledge that can be incorporated into breeding programmes for improving the natural resistance to diseases in tropical environments. Recently, we described different levels of CPW and APW between non-immunized birds from various ecotypes (Baelmans *et al*., in press). In the present study, we measured the haemolytic activity of both classical and alternative complement pathways at 7 days after immunization with SRBC as a measure of complement consumption or stimulation. We also evaluated the effect of major genes for feather reduction (*Nana*$^{-}$), feather curling and reduction (*Ff*$^{-}$) and body reduction (*dw*$^{-}$) on antibody responses and complement activity. These major genes are known to improve heat endurance (Garces *et al*., 2001), and are often implemented in breeding programmes with local chickens to increase poultry production.

MATERIALS AND METHODS

Chickens

Indigenous chickens of various breeds and origins were separated by geographical locations and referred to as ecotypes. A total of 248 clinically healthy adult indigenous hens from 18 different locations (Table I) from Bolivia, India and Tanzania were conventionally raised on the floor and fed *ad libitum* with local commercial diets at the local universities. In Bolivia, 89 adult scavenging local chickens from seven Andean regions were purchased and kept at the University of Oruro (Universidad Technica de Oruro). In Tanzania, 102 indigenous chickens were purchased from seven different eco-climatic regions from all over the country. Eighty-six hens were housed at the Central Avian Research Institute (CARI, Izatnagar, India). The following lines were studied: the Yellow Aseel, an indigenous 'fighting' breed from Andhra Pradesh; the Kadaknath, a famous Indian indigenous breed used for medicinal purposes in tribal areas; the Indian frizzled typed; and the Indian naked neck broiler-type chickens. At least 2 months before the experiment, all hens were vaccinated against Newcastle disease, treated against ectoparasites and wing-tagged.

Day-old GDR chicks were kindly provided by Professor P. Horst and were transported from the Institute of Animal Sciences, Humboldt University of Berlin, Germany to the Institute of Tropical Medicine, Department of Animal Health, Antwerp, Belgium. The pullets were the offspring of a Dahlem Red experimental male line heterozygous for the naked neck (*Nana*$^{-}$), frizzle (*Ff*$^{-}$) and dwarf (*dw*$^{-}$) genes and a Rhode Island White female line homozygous for the normal alleles of the three genes. Eight different combinations of genes for body size and feather coverage, constituting eight different genetic groups of 16 individuals, were segregated (Table II). The birds were vaccinated for Marek's disease on day 0 of age and subjected to intramuscular SRBC immunization at 9 weeks of age.

TABLE I
Source of chickens from different countries and different continents

Country	Ecotype	Number of birds
Bolivia	Alk'arapi	6
Bolivia	Altiplano	10
Bolivia	Chuhuica	9
Bolivia	Iroko	6
Bolivia	K'oma	6
Bolivia	Oruro	16
Bolivia	Pollok'eri	7
India	Aseel (Yellow)	24
India	Indian frizzle (Ff^{-}) type	24
India	Indian naked neck ($Nana^{-}$) type (broiler)	19
India	Kadaknath	19
Tanzania	Arusha	17
Tanzania	Coast region	12
Tanzania	Mbeya	14
Tanzania	Moshi	8
Tanzania	Mwanza	10
Tanzania	Singida	33
Tanzania	Songea	8

TABLE II
Eight German Dahlem Red (GDR) chicken lines with major genes

Genetic specification	Phenotypic classification	Number of birds
nanaffDw^{-}	Control line Dahlem Red (exotic)	16
nanaffdw^{-}	Dwarf line Dahlem Red	16
NanaffDw^{-}	Naked neck Dahlem Red	16
Nanaffdw^{-}	Naked neck and dwarf	16
nanaFfDw^{-}	Frizzle Dahlem Red	16
nanaFfdw^{-}	Frizzle dwarf	16
NanaFfDw^{-}	Frizzle and naked neck	16
NanaFfdw^{-}	Frizzle, naked neck and dwarf characteristics	16

Blood collection

All chickens were bled by the wing vein before SRBC immunization (to assess initial values of non-specific antibodies to SRBC and initial complement levels), and 7 days thereafter. Serum samples were kept at –20°C until use. SRBC were collected either from animals slaughtered at an abattoir in Antwerp or from sheep at the local universities. The blood was collected in Alsever solution (1:1 v/v). On the day of immunization, SRBC were washed three times with phosphate buffered saline (PBS, pH 7.2) and administered to the chickens intramuscularly at a dose of 0.5 ml 50% (packed) SRBC in the breast muscles using a 2 ml syringe with a 21G needle.

Immunological tests

Haemolytic complement activity: Classical calcium-dependent (CPW) and alternative calcium-independent (APW) activities were determined with a haemolytic technique as described elsewhere (Demey *et al.*, 1993; Parmentier *et al.*, 2002) using an adapted light-scattering method. Complement activity was expressed as delta (δCH_{50} U/ml): being the difference between initial complement levels minus the complement levels at 7 days post immunization (PI) with SRBC.

Haemagglutination: Primary antibody titres to SRBC, either IgTotal or mercapto-ethanol-resistant (MER), representing the IgG isotype, were determined by a haem-agglutination assay (Parmentier *et al.*, 2002). (Delta) Antibody titres measured against SRBC were expressed as the $\log_2$ of the reciprocal of the highest serum dilution giving complete agglutination at day 7 PI minus day 0, using the same SRBC that were used for the immunizations. Mercaptoethanol sensitive antibodies (MES), representing the IgM isotype, are calculated as the difference between IgTotal antibody titres and MER titres.

Statistics

Statistical analyses were carried out in Stata 8 (StataCorp., 2003). The means of the different (delta) antibody titres and classical and alternative complement activity were compared by means of a one-way analysis of variance, followed by a Scheffé multiple comparison test in the case of a significant *F*-value. Bartlett's test was used to verify equality of variances. The correlations between the different (delta) antibody titres and CPW or APW complement activities were tested by means of Spearman's rank correlation, using Bonferroni-adjusted probabilities to account for the number of tests performed.

RESULTS

Humoral immune response to SRBC immunization

The distributions of delta (day 7 – day 0) IgTotal, MER and MES antibody titres from various ecotypes are shown in Table III. In all ecotypes studied, a wide variation in total antibody responses to SRBC immunization was found, from no response (various breeds from Bolivia) up to 12 $\log_2$ (Indian Ff$^-$) at day 7 PI (Figure 1). Antibody responses of indigenous Bolivian chickens to SRBC were low. Bolivian hens belonging to the Pollok'eri ecotype had the lowest IgTotal and MER (IgG) antibody responses to SRBC. The majority (49%) of Bolivian hens were not capable of producing MER antibodies (IgG) to SRBC at 7 days PI (0 $\log_2$). From the Tanzanian indigenous chickens, those from the Moshi region had the highest IgTotal and MER (IgG) antibody responses, while the Mwanza ecotype showed the lowest IgTotal and MER antibody responses. From the Indian breeds, the highest IgTotal antibody titres were found in the Kadaknath line, mainly consisting of MES (IgM) antibodies. Between the four Indian lines tested, the naked neck (Nana$^-$) line showed the highest antibody response (IgTotal and MES) to SRBC, whereas the Indian Ff$^-$ line had the highest MER response at 7 days PI. GDR lines had higher MER (IgG) antibody production

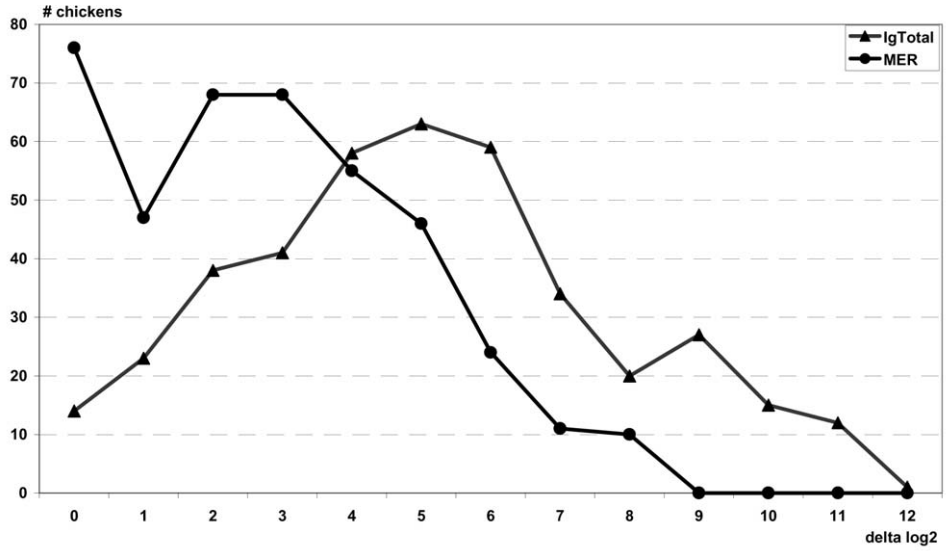

Figure 1. Distribution of δIgTotal and δMER (IgG) titres of 376 SRBC-immunized chickens at day 7 PI

TABLE III
Delta antibody response (day 7 – day 0) to SRBC immunization of indigenous hens (**B**, Bolivian origin; **T**, Tanzanian origin; **I**, Indian origin) and GDR hens with major genes (mean $\log_2 \pm$ SE)

Ecotype	IgTotal	Ecotype	MER	Ecotype	MES
Pollok'eri **B**	1.64 ± 0.63^{a}	Pollok'eri **B**	0.29 ± 0.19^{a}	K'oma **B**	0.25 ± 0.17^{a}
Iroko **B**	2.25 ± 0.54^{ab}	Alk'arapi **B**	0.58 ± 0.27^{ab}	Nana ffDw^{-}	0.81 ± 0.17^{a}
Alk'arapi **B**	2.33 ± 0.80^{ab}	Iroko **B**	1.17 ± 0.65^{ab}	Nana ffdw^{-}	1.03 ± 0.16^{a}
K'oma **B**	2.33 ± 0.49^{ab}	Mwanza **T**	1.20 ± 0.42^{ab}	Iroko **B**	1.08 ± 0.49^{ab}
Altiplano **B**	2.90 ± 0.27^{ab}	Altiplano **B**	1.20 ± 0.33^{ab}	Chuhuica **B**	1.11 ± 0.48^{ab}
Chuhuica **B**	3.00 ± 0.51^{ab}	Songea **T**	1.75 ± 0.66^{ab}	nana ff Dw^{-}	1.13 ± 0.19^{ab}
nana ff Dw^{-}	3.50 ± 0.46^{ab}	Chuhuica **B**	1.78 ± 0.62^{ab}	nana ff dw^{-}	1.13 ± 0.32^{ab}
Mwanza **T**	3.60 ± 0.58^{ab}	nana Ff Dw^{-}	2.06 ± 0.43^{ab}	Pollok'eri **B**	1.36 ± 0.50^{abc}
Oruro **B**	3.75 ± 0.40^{ab}	K'oma **B**	2.08 ± 0.61^{ab}	Oruro **B**	1.44 ± 0.42^{abc}
nana Ff Dw^{-}	3.75 ± 0.41^{ab}	Arusha **T**	2.12 ± 0.54^{ab}	nana Ff dw^{-}	1.56 ± 0.32^{abc}
Nana ff Dw^{-}	3.88 ± 0.43^{ab}	Coast **T**	2.17 ± 0.42^{ab}	Nana Ff dw^{-}	1.59 ± 0.28^{abc}
Arusha **T**	4.06 ± 0.50^{ab}	Oruro **B**	2.31 ± 0.48^{ab}	Nana Ff Dw^{-}	1.69 ± 0.23^{abc}
Coast **T**	4.42 ± 0.51^{abc}	Singida **T**	2.33 ± 0.27^{ab}	nana Ff Dw^{-}	1.69 ± 0.22^{abc}
Nana ff dw^{-}	4.69 ± 0.62^{abc}	nana ff Dw^{-}	2.38 ± 0.49^{ab}	Altiplano **B**	1.70 ± 0.40^{abc}
Singida **T**	4.85 ± 0.33^{abc}	Mbeya **T**	3.00 ± 0.30^{ab}	Alk'arapi **B**	1.75 ± 0.68^{abcd}
Nana Ff dw^{-}	5.38 ± 0.49^{abcd}	Aseel **I**	3.00 ± 0.29^{ab}	Arusha **T**	1.94 ± 0.33^{abcd}
Aseel **I**	5.50 ± 0.36^{abcd}	Nana ff Dw^{-}	3.06 ± 0.45^{ab}	Coast **T**	2.25 ± 0.46^{abcd}
Songea **T**	5.63 ± 0.63^{abcd}	Kadaknath **I**	3.18 ± 0.28^{ab}	Moshi **T**	2.38 ± 0.57^{abcd}
Mbeya **T**	5.64 ± 0.41^{abcd}	Indian Nana^{-}I	3.47 ± 0.49^{ab}	Mwanza **T**	2.40 ± 0.37^{abcd}
Nana Ff Dw^{-}	5.84 ± 0.67^{abcd}	Moshi **T**	3.50 ± 0.50^{ab}	Aseel **I**	2.50 ± 0.35^{abcd}
Moshi **T**	5.88 ± 0.70^{abcd}	Nana ff dw^{-}	3.66 ± 0.60^{ab}	Singida **T**	2.52 ± 0.31^{abcd}
nana ff dw^{-}	6.13 ± 0.39^{abcd}	Nana Ff dw^{-}	3.78 ± 0.64^{ab}	Mbeya **T**	2.64 ± 0.54^{abcd}
nana Ff dw^{-}	7.13 ± 0.54^{bcd}	Indian Ff^{-} **I**	3.79 ± 0.26^{ab}	Songea **T**	3.88 ± 0.70^{abcd}
Indian Ff^{-} **I**	8.19 ± 0.36^{cd}	Nana Ff Dw^{-}	4.16 ± 0.64^{ab}	Indian Ff^{-} **I**	4.40 ± 0.29^{bcd}
Indian Nana^{-}I	8.45 ± 0.58^{cd}	nana ff dw^{-}	4.94 ± 0.57^{ab}	Indian Nana^{-}I	4.97 ± 0.39^{cd}
Kadaknath **I**	8.89 ± 0.36^{d}	nana Ff dw^{-}	5.56 ± 0.48^{b}	Kadaknath **I**	5.71 ± 0.26^{d}

[abcd]The same letter indicates absence of a significant difference between the respective averages

MER, mercaptoethanol-resistant antibodies, representing IgG

MES, IgTotal minus mercaptoethanol-resistant antibodies, representing IgM

compared to indigenous birds. No significant differences were observed between the eight GDR lines with major genes in humoral responses (IgTotal, MER and MES) to SRBC. A highly significant ($p < 0.001$) correlation was calculated between IgTotal and MER (IgG) and between IgTotal and MES (IgM) antibody titres to SRBC ($r = 0.71$ and $r = 0.54$, respectively) and a negative correlation between MER and MES antibody titres to SRBC ($r = -0.16$, $p = 0.022$).

Haemolytic complement activity after SRBC immunization

The data on complement activity after SRBC immunization varied more between individual chickens than between ecotypes. Haemolytic calcium-independent APW complement activity decreased in 46% of the indigenous chicken population from different ecological zones, i.e. higher APW levels were found at 7 days PI with SRBC than prior to immunization. Most of the decreased APW activity at day 7 PI was found in indigenous Bolivian and Indian chickens (Table IV).

A comparison of complement activity (calcium-dependent (CPW) and calcium-independent (APW)) in the indigenous hens from Bolivia in response to SRBC immunization revealed no significant differences.

Within the Bolivian or the Tanzanian breeds, no significant differences were found in the CPW activity after SRBC immunization, suggesting that geographical location has no influence on immune competence between ecotypes. Most chickens from the region of Songea showed a decrease in CPW activity in response to SRBC, and most of the Tanzanian hens showed an increase in CPW but a decrease in APW activity after immunization with SRBC. All Indian chicken lines showed a high CPW activity in response to SRBC. All individuals with a CPW activity higher than 1400 CH_{50} U/ml (n = 27) (Figure 2) belonged to the Indian lines. The Indian naked neck line had the highest CPW activity in response to SRBC and is significantly different from the Indian Yellow Aseel. The Yellow Aseel differed significantly from the Indian Ff^- and the Indian $Nana^-$ birds in APW activity. Both lines (Indian Ff^- and $Nana^-$) had a major decrease in APW activity, whereas their CPW activity was one of the highest.

No significant effects of the presence or absence of the frizzled (Ff^-) and/or naked neck ($Nana^-$) and/or dwarfism (dw^-) genes on complement activity (consumption) to SRBC in the GDR lines were evident. Between the GDR genotypes, nana Ff Dw^- was a low complement responder compared to the high complement responders of phenotypes nana ff dw^- and nana Ff dw^-.

Significative correlations were demonstrated between (delta) IgTotal antibody titres and CPW activity ($r = 0.57$, $p < 0.001$) and a low correlation between CPW activity and APW activity ($r = 0.16$, $p < 0.020$). No correlation ($r = 0.04$) was found between (delta) IgTotal antibody titres to SRBC and APW activity.

DISCUSSION

In the present study to establish immune competence of various ecotypes, specific antibody responses to SRBC and the activity of both the classical (CPW) and the alternative (APW) complement pathways were evaluated after immunization with SRBC and the effects of major genes on the response of specific and innate immune responses was determined. Complement components and complement receptors belonging to the classical and alternative pathways, which precede the common terminal (lytic) pathway, are major constituents of innate immunity. Components of the complement system are involved in the initiation of specific antibody responses, antigen trapping in lymphoid germinal centres, development and maintenance of

TABLE IV
Haemolytic complement activity (CPW and APW) at 7 days pi with SRBC of indigenous hens (**B**, Bolivian origin; **T**, Tanzanian origin; **I**, Indian origin) and GDR hens with major genes (mean CH_{50} U/ml ± SE)

ecotype	δCPW	Ecotype	δAPW
Songea **T**	17 ± 136[a]	Indian Ff⁻ **I**	–236 ± 42[a]
K'oma **B**	67 ± 31[ab]	Arusha **T**	–166 ± 55[ab]
Pollok'eri **B**	95 ± 48[ab]	Indian Nana⁻ **I**	–118 ± 47 [abc]
Altiplano **B**	100 ± 14[ab]	Songea **T**	–37 ± 41[abcd]
Iroko **B**	102 ± 21[ab]	Pollok'eri **B**	–30 ± 13[abcd]
Alk'arapi **B**	111 ± 28[ab]	Mbeya **T**	–22 ± 36[abcd]
Chuhuica **B**	118 ± 45[ab]	Oruro **B**	9 ± 11[abcd]
Oruro **B**	181 ± 37[ab]	Iroko **B**	15 ± 42[abcde]
Arusha **T**	202 ± 103[ab]	Moshi **T**	31 ± 34[bcde]
Mwanza **T**	238 ± 71[abc]	K'oma **B**	53 ± 19[bcde]
nana Ff Dw⁻	278 ± 50[abc]	Kadaknath **I**	57 ± 23[bcde]
Singida **T**	341 ± 46[abc]	Singida **T**	69 ± 20[bcde]
Coast **T**	406 ± 85[abcd]	Chuhuica **B**	90 ± 45[bcde]
nana ff Dw⁻	422 ± 51[abcd]	Coast **T**	99 ± 28[bcde]
Mbeya **T**	440 ± 48[abcd]	Altiplano **B**	109 ± 34[bcde]
Nana Ff dw⁻	453 ± 97[abcd]	Alk'arapi **B**	122 ± 64[bcde]
Moshi **T**	464 ± 123[abcde]	nana Ff Dw⁻	123 ± 26[cde]
Nana ff Dw⁻	473 ± 46[abcde]	Nana ff dw⁻	128 ± 30[cde]
Nana Ff Dw⁻	481 ± 54[abcde]	Mwanza **T**	137 ± 41[cde]
Nana ff dw⁻	538 ± 75[abcde]	Nana ff Dw⁻	179 ± 19[de]
nana Ff dw⁻	584 ± 70[bcde]	nana ff Dw⁻	180 ± 42[de]
nana ff dw⁻	586 ± 58[bcde]	Nana Ff dw⁻	210 ± 34[de]
Yellow Aseel **I**	756 ± 193[cde]	Nana Ff Dw⁻	221 ± 41[de]
Kadaknath **I**	964 ± 173[def]	nana ff dw⁻	268 ± 46[de]
Indian Ff⁻ **I**	1088 ± 198[ef]	nana Ff dw⁻	331 ± 39[e]
Indian Nana⁻ **I**	1643 ± 221[f]	Yellow Aseel **I**	340 ± 75[e]

[abcdef]The same letter indicates absence of a significant difference between the respective averages

δCPW, haemolytic classical complement activity at 7 days PI with SRBC minus haemolytic classical complement activity at day 0

δAPW, haemolytic alternative complement activity at 7 days PI with SRBC minus haemolytic alternative complement activity at day 0

memory cells and switching of IgM to IgG isotypes (Thorbecke *et al.*, 1994). Earlier we described higher levels of (the antibody-dependent) CPW in chickens that had been divergently selected for antibody responses to SRBC (Parmentier *et al.*, 2002). We also found striking differences in the levels of both CPW and APW between local chicken breeds (ecotypes) worldwide. The lowest CPW and APW levels were found in the Bolivian ecotypes in the present study, whereas breeds from Tanzania, Benin and

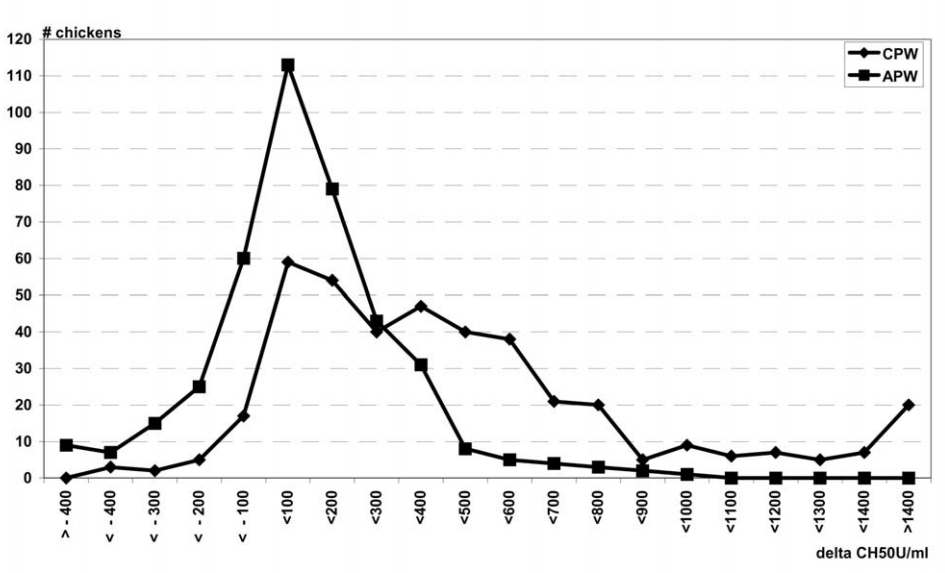

Figure 2. Distribution of δCPW and δAPW activity (day 7 – day 0 complement levels) of chickens at day 7 PI with SRBC

Cameroon had highest CPW and APW levels (Baelmans *et al.*, in press). Levels of CPW and APW of most ecotypes were significantly lower than levels of CPW and APW as found in 'commercial' Leghorn breeds (Parmentier *et al.*, 2004), suggesting effects of both genetic background and environment (husbandry). In ecotypes, as well as 'commercial' chicken lines, high ranking of CPW levels of non-immunized birds was not always accompanied by high ranking of APW levels, suggesting different genetic regulation of CPW versus APW and different effects of the environment on CPW and APW levels. The present data extended these findings. We found different CPW and APW activities after immunization with SRBC between the various ecotypes. Low CPW activity was found in the Bolivian ecotypes, whereas the highest CPW activity was found in the Indian breeds, and intermediate levels of CPW activity in the African birds. There was a consistent discordance between the CPW activities (δCPW) and APW activities (δAPW) in response to immunization with SRBC in indigenous chickens, especially in the Indian Ff^{-} and $Nana^{-}$ lines. Both ecotypes showed high CPW (and humoral) responses but showed a large fall in APW activity. The low correlation between CPW and APW activity between the various ecotypes suggests differential activation of the CPW and APW pathways in the various ecotypes.

The ecotypes could be differentiated by their IgTotal antibody responses and isotype (IgG and IgM) related antibody responses to SRBC. Again, the lowest responses were found in the Bolivian ecotypes (IgTotal, IgM and IgG), and the highest responses in the

Indian ecotypes (IgTotal and IgM) and GDR breeds with major genes (IgTotal and IgG). Differentiating the primary antibody response into mainly IgG (δMER) and IgM (δMES) showed that the response in the GDR hens was mainly of IgG isotype, whereas the indigenous Indian hens' humoral response rested on IgM production to SRBC at 7 days PI. No significant difference occurred in antibody responses (δTotal, δMER and δMES) among the native Indian lines. Similar results were obtained by Kundu and colleagues (1999). In the present study, the nana ff Dw^{-} Dahlem Red breed showed significantly lower IgTotal antibody responses than the Indian lines (except Yellow Aseel) and lower IgM responses than the Kadaknath and Indian $Nana^{-}$ hens. This suggests that indigenous Indian chicken populations showed superior immune competence with respect to primary antibody responses to SRBC (Kundu *et al.*, 1999). The major genes of tropical interest within the GDR chickens also had lesser effect on the humoral response to SRBC, which is in accordance with Haunshi and colleagues (2002). Hens carrying different combinations of major genes ($Nana^{-}$, dw^{-}, Ff^{-}) also did not show different complement responses to immunization with SRBC. Earlier, we determined different CPW and APW levels in these lines (Dorny *et al.*, in press). However, all GDR lines had higher complement activities than the Tanzanian and Bolivian ecotypes.

The positive correlation between CPW activity and IgTotal, IgG and IgM antibodies, respectively, the negative correlation between IgM and IgG responses, and the lack of correlation between antibody responses and APW suggested four types of immune responsiveness towards SRBC immunization. These types of responsiveness included: no specific (antibody) or innate (APW) responsiveness (most Bolivian breeds); IgM-specific and high CPW responsiveness (Tanzanian and Indian ecotypes); IgG-specific and high CPW responsiveness (GDR birds); and no specific and low to moderate APW responsiveness (Bolivian breeds). These results indicate that type and magnitude of immune responsiveness depend on breed and local environment. However, the data on immune competence varied more between individual chickens than between ecotypes, as exemplified by the Tanzanian breeds, and no ecotype was significantly superior to the others regarding humoral responses to SRBC. These findings are in agreement with Msoffe and colleagues (2001). The diversity of the repertoire of primary antibody (isotype) responses and complement within ecotypes may be as great as that between ecotypes (Sonaiya *et al.*, 1998). Such diversity may account for the variability in immune responsiveness of ecotypes, which is presumably required to combat a variety of infectious diseases.

In conclusion, we established the existence of large individual variations in complement and antibody responses after immunization of indigenous chickens with SRBC. The high correlation between antibody titres to SRBC (including IgM and IgG antibodies) but low correlation between CPW and APW with respect to haemolytic activity and the absence of correlation between antibody responses and APW suggest that measurement of either CPW and APW levels may give valuable information on both specific and innate immune responsiveness. Whether the differences in antibody titres, CPW and APW reflect local hygienic conditions and affect disease susceptibility remains to be established.

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Activité du complément hémolytique et réponses immunes humorales aux globules rouges du mouton chez des poulets indigènes et chez huit lignées de poulets allemands Dahlem Red ayant différentes combinaisons de gènes majeurs (nain, cou nu et frisé) d'intérêt tropical

Résumé – Un total de 376 poulets de différents écotypes ont été immunisés avec des globules rouges de moutons à antigène multidéterminant non-pathogénique (SRBC). Les écotypes ont inclus des poulets indigènes provenant de diverses régions de Tanzanie (n = 102), d'Inde (n = 86) et de Bolivie (n = 89). De surcroît, huit lignées de poulets allemands Dalhem Red possédant différents gènes majeurs (nain, cou nu et frisé) présentant un intérêt tropical ont également été immunisés avec les SRBC. La compétence immunitaire des races a été évaluée en mesurant l'activité du complément hémolytique, à la fois à partir de la voie classique du complément dépendant du calcium (CPW) et de la voie alternative du complément indépendant du calcium (APW), et également les réponses aux anticorps IgTotal, IgG et IgM aux SRBC 7 jours après l'immunisation. Des variations considérables des réponses des anticorps et de l'activité du complément aux SRBC ont été observées au sein et entre les races indigènes. De nombreux poulets indigènes, en particulier en provenance de Bolivie, ont manifesté une diminution de l'activité du complément (APW) suite à une immunisation avec les SRBC. Les races en provenance d'Inde ont manifesté une activité du CPW et des réponses humorales (en particulier de l'IgM) aux SRBC les plus élevées, ce qui suggère une compétence immunitaire élevée. Par contraste, les poulets boliviens caractérisés par une basse activité du CPW, une faible activité de l'APW et de faibles taux d'anticorps aux SRBC suggèrent une compétence immunitaire globalement basse. Chez les poulets allemands Dalhem Red, caractérisés par une activité du CPW élevée et des réponses élevées des anticorps IgG aux SRBC, les gènes majeurs cou nu, frisé et nain n'ont manifesté aucun effet significatif sur les réponses des anticorps et l'activité du complément aux SRBC.

Actividad hemolítica del complemento y respuestas inmune humorales a glóbulos rojos de ovejas en pollos indígenas y en ocho líneas de pollos rojos alemanes Dahlem con diferentes combinaciones de genes principales (enano, de cuello desnudo y rizado) de interés tropical

Resumen – Un total de 376 pollos de diferentes ecotipos fueron inmunizados con el antígeno no patogénico y multi-determinante de glóbulos rojos de ovejas (SRBC, en inglés). Los ecotipos incluían pollos indígenas de distintas localizaciones de Tanzania (n = 102), India (n = 86) y Bolivia (n = 89). Además, fueron también inmunizados con SRBC ocho líneas de pollos de raza roja alemana Dahlem (GDR, en inglés) con diferentes genes principales (enano, cuello desnudo y rizado) de interés tropical. La competencia inmunológica de las estirpes fue evaluada midiendo la actividad hemolítica del complemento, tanto de la ruta de complemento clásica dependiente de calcio (CPW) como de la ruta alternativa independiente de calcio (APW), además de las respuestas de anticuerpos de IgTotal, IgG y IgM a SRBC a los 7 días después de la inmunización. Se observaron grandes variaciones en la actividad del complemento y en las respuestas de anticuerpo a SRBC dentro y entre las razas indígenas. Muchos pollos indígenas, especialmente de Bolivia, mostraron una actividad del complemento menor (APW) después de la inmunización con SRBC. Las razas o estirpes de la India mostraron la actividad CPW y respuestas humorales (especialmente IgM) más altas a SRBC, sugiriendo con ello una alta competencia inmunológica. Por el contrario, los pollos bolivianos estuvieron caracterizados por baja actividad CPW, baja actividad APW, y bajos niveles de anticuerpo a SRBC, sugiriendo con ello una competencia inmunológica baja en general. En los pollos GDR, caracterizados por alta actividad CPW y altas respuestas de anticuerpo IgG a SRBC, los genes principales para cuello desnudo, rizamiento y enanismo no tuvieron un efecto significativo en las respuestas de anticuerpo y actividad del complemento a los glóbulos rojos de ovejas (SRBC).