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Different Serum Haemolytic Complement Levels in Indigenous Chickens from Benin, Bolivia, Cameroon, India and Tanzania

R. Baelmans[1], H.K. Parmentier[2]*, H.M.J. Udo[3], P. Dorny[1], F. Demey[1] and D. Berkvens[1]
[1]*Institute of Tropical Medicine, Department of Animal Health, Antwerp, Belgium;*
[2]*Adaptation Physiology Group,* [3]*Animal Production Systems Group, Department of Animal Sciences, Wageningen University, Marijkeweg 40, 6709 PG Wageningen, The Netherlands*
**Correspondence: E-mail: Henk.Parmentier@WUR.nl*

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ABSTRACT

Titres of classical (CPW) and alternative (APW) complement pathways were measured in clinically healthy local chicken populations (ecotypes) from Africa (Benin, n = 78; Cameroon, n = 299; Tanzania, n = 101), Asia (India, n = 96) and South America (Bolivia, n = 64). A wide variation was found in haemolytic complement levels between the various ecotypes. Distributions of the classical and alternative complement titres were not normal but were skewed to the right. Differences in complement were found both within and between ecotypes. Furthermore, CPW titres of the indigenous chickens were lower than those determined in commercial layer chickens. This suggests that complement levels in indigenous hens are not solely dependent on local antigenic pressure but may also depend on genetic background and husbandry. The relationships between complement levels, the chicken MHC(B) complex, environmental antigenic pressure, and survival of the scavenging local chickens are discussed.

Keywords: complement, chicken, indigenous, ecotypes, Cameroon, Benin, Bolivia, India, Tanzania

Abbreviations: APW, alternative (calcium-independent) pathway; CH_{50}, titre that lyses 50% of erythrocytes; CPW, classical (calcium-dependent) pathway; MHC, major histocompatibility (B) complex

INTRODUCTION

Chickens (*Gallus domesticus*) dominate the smallholder production systems of developing countries. Traditional poultry systems constitute 80% of the world poultry population, which consists of approximately 14 billion birds. Chickens are of great importance for village households in developing countries because they provide a source of protein (eggs and meat) with minimal input. In general, village producers keep small flocks of between 4 and 20 chickens per household. Women and children play a key role in their management (Bradley, 1992; Kitalyi, 1997; Udo, 1997; Dolberg, 2001). The chickens are generally raised in a free-range system and scavenge around the compound where they feed on the local feedstuff and garbage (Kitalyi, 1997; Spradbrow, 1997; Udo, 1997; Dolberg, 2001). For centuries this low input/output practice has been a traditional component of small farms all over the world, and this will probably continue (Kitalyi, 1997; Spradbrow; 1997). Poultry diseases seriously affect village chicken production. It is estimated that mortality of indigenously managed chickens is about 70% or higher up to 8 weeks of age (Sonaiya, 1990; Spradbow, 1994; Okitoi *et al.*, 1999). Indigenous chickens are well adapted to environmental stress and contain a highly conserved polygenetic system, reflecting native genetic diversity (Wimmers *et al.*, 2000). Whatever stress affects the individuals in the populations, natural selection during the course of breeding will improve, or at least maintain, direct resistance to that stress, or indirectly to other stresses. Such conservation is intuitively logical, owing to the ability of these chickens to survive stresses for thousands of generations without proper nutrition and vaccination, largely because these are too costly for developing countries (Udo, 1997). The main poultry breeding companies, on the other hand, have been doing just the opposite: applying optimal management and biosecurity for their selection flocks, leading to a (very) narrow genetic diversity, which may lead to a further increase in sensitivity to climatic and other stresses (Pinard-van der Laan, 2002).

The immune system of chickens is influenced by several external (climatic, social and microbial) factors. To understand and enhance avian immune competence in indigenous populations and to improve productivity and health of poultry under village conditions, multidisciplinary approaches are required (Sonaiya *et al.*, 1999). The classical and alternative complement pathways belong phylogenetically to the oldest immune defences of vertebrates. Study of the haemolytic serum complement titres (the classical calcium-dependent pathway (CPW) and the alternative calcium-independent pathway (APW)), which constitute major first lines of defence of indigenous chickens, is one of those multidisciplinary approaches (Pandey *et al.*, 1992). Knowledge of complement levels (and dynamics or consumption) may contribute to a comprehensive strategy to improve innate resistance to diseases and enhance immune competence of poultry.

We recently described CPW and APW titres in various commercial layer lines and the relation with the major histocompatibility (B) complex of the lines (Parmentier *et al.*, 2002; Parmentier *et al.*, 2004). In the present study we investigated complement titres in indigenous chickens of various breeds and origin, separated by geographical locations, referred to as ecotypes.

MATERIALS AND METHODS

Chickens

Blood was collected from adult, clinically healthy indigenous hens from Cameroon, Benin, Tanzania, India and Bolivia. In Cameroon, sampling was done on 299 local chickens in the area of Dschang, about 250 km north-east of Douala. The backyard chickens were taken from four different regions: Balepie (*n* = 65), Bafou (*n* = 39), Foto (*n* = 21) and Fongo-Tongo (*n* = 39). In addition, a total of 135 indigenous chickens were sampled at the university campus of Dschang (CUDS). Blood samples were taken from 78 local chickens originating from the southern region of Benin, at the National University of Benin (UNB), Cotonou. In Tanzania, 101 local chickens were purchased from seven different regions across the country: Moshi (*n* = 6), Songea (*n* = 7), Mbeya (*n* = 16), Arusha (*n* = 15), Mwanza (*n* = 11), Singida (*n* = 34) and the Coast region (*n* = 12) of Tanzania. They were examined and blood was sampled at the Sokoine University of Agriculture (SUA), Morogoro, Tanzania. In India, a total of 96 hens from the four most common North Indian 'backyard' chicken lines were sampled: the Yellow Aseel (*n* = 25), a indigenous 'fighting' breed from Andhra Pradesh; the Kadaknath (*n* = 23), a famous Indian indigenous breed used for medicinal purposes in tribal areas; the Indian frizzled typed (*n* = 24); and the Indian naked neck ($Nana^{-}$) broiler typed chickens (*n* = 24). The last two breeds are both highly adapted to heat stress. The birds were housed at the Central Avian Research Institute (CARI, Izatnagar). In Bolivia, a total of 64 local chickens were purchased from seven different Andean regions (at altitudes between 3600 and 4000 m): Alk'arapi (*n* = 6); Pollok'eri (*n* = 7); K'oma (*n* = 6); Chuhuica (*n* = 9), Iroko (*n* = 6), altiplano in general (*n* = 10); and 20 individuals from the northern part of Oruro. The latter birds were kept at the University of Oruro (Universidad Technica de Oruro, UTO).

Blood collection

All chickens were bled by the wing vein and blood was collected in ice-cold glass tubes without anticoagulant. The blood was put on ice and individual sera were stored within 6 h at –20°C (or at –70°C where available). All serum samples were examined for complement levels within one month after blood collection.

Complement assays

Complement activity was determined with a haemolytic technique (Demey *et al.*, 1993) using an adapted light-scattering method. In brief, sera were diluted serially in appropriate (Ca^{2+}-containing or Ca^{2+}-depleted) buffers in flat-bottomed 96-well microtitre plates and incubated with sensitized (Haemolysin, Biomerieux, ref. no. 72202) sheep erythrocytes for measurement of CPW or rabbit erythrocytes for measurement of APW. Plates were shaken in a Titertek (Flow Laboratories) every 30 min during the period of incubation. The results (the amount of light scattering by erythrocytes upon lysis) were read at 655 nm in a microtitre reader (BioRad model 3550). Readings were log–log transformed (Von Krogh, 1916) and the haemolytic titre was expressed as the titre that lysed 50% of the erythrocytes (CH_{50} U/ml).

Statistical analysis

Statistical analyses were carried out using Stata 8 (StataCorp., 2003). Mean CPW and APW values were compared using negative binomial regression. Probability levels were Bonferroni-adjusted to account for the large number of comparisons made. Clustering and dendrogram construction were done using Euclidean distance and hierarchical agglomerative average linkage.

RESULTS

Classical complement titres

Distribution of classical complement (CPW) titres in indigenous chickens is shown in Table I. The highest titres of CPW were found in the Yellow Aseel breed (India) and in local populations in Benin and Tanzania (Moshi), respectively. A wide variation in CPW levels was found in these ecotypes. On the other hand, the Bolivian ecotypes showed much lower CPW titres ranging from 47 to 277 CH_{50} U/ml. The levels of CPW from the Bolivian birds and from two ecotypes of Cameroon (Bafou, Foto) differed significantly from the CPW titres from Indian, Tanzanian and Benin chickens. A wide variation of the CPW titres was found between the Cameroon populations. Hens from the Foto and Bafou regions had significantly lower CPW titres than hens from the Balapie, Dschang and Fongo-tongo regions, respectively. Within the Indian lines, significant differences were found between the high CPW titres of Yellow Aseel birds and the low levels of the Kadaknath and naked neck birds. Frizzled (Ff^-) birds also differed significantly from the Indian $Nana^-$ birds and the Indian black hens. With respect to the Tanzanian chickens, birds from the Moshi and Songea regions showed the highest CPW titres. The CPW titres of the chickens from the Moshi region were significantly higher than the CPW titres from chickens from the Mbeya, Mwanza, Singida and the Coast region.

Alternative complement titres

The distribution of APW titres in indigenous chickens is shown in Table I. The highest average APW titres were found in the Benin birds, followed by various ecotypes from Cameroon. Titers of APW from Tanzanian lines were in the middle. Similarly, as for the CPW, average APW titres were in general low in the Bolivian populations. The lowest APW titres within the Indian lines were the Kadaknath hens. Numerous Benin birds showed APW titres higher than 1000 CH_{50} U/ml (highest level up to 3508 CH_{50} U/ml). APW titres of Benin birds were significantly higher than APW titres of Bolivian chickens. Significant differences between the low APW titres of the Kadaknath line and the other Indian lines were found. The intermediate APW titres of the Indian Aseel birds were also significantly different than the APW titres of the Indian Ff^- birds. No significant differences between APW titres of the birds from the various Cameroonian ecotypes were found.

TABLE I
Indigenous hens and their mean complement titres (CH_{50} U/m ±SEM)

Ecotype	Calcium-dependent pathway	Ecotype	Calcium-independent pathway
Alk'arapi (B)	110 ± 4^{a}	Alk'arapi (B)	107 ± 17^{a}
Oruro (B)	112 ± 5^{ab}	Kadaknath (I)	123 ± 17^{a}
Pollok'eri (B)	113 ± 14^{abc}	Mwanza (T)	134 ± 19^{ab}
Iroko (B)	124 ± 11^{abc}	Oruro (B)	138 ± 13^{ab}
Altiplano (B)	125 ± 5^{abc}	Pollok'eri (B)	140 ± 10^{abc}
K'oma (B)	137 ± 22^{abcd}	K'oma (B)	167 ± 25^{abcd}
Chuhuica (B)	148 ± 20^{bcd}	Coast (T)	169 ± 25^{abcd}
Bafou (C)	154 ± 7^{cd}	Iroko (B)	174 ± 25^{abcd}
Foto (C)	167 ± 17^{d}	Altiplano (B)	181 ± 21^{abcd}
Mbeya (T)	266 ± 34^{e}	Singida (T)	184 ± 12^{abcd}
Indian Nana- (I)	268 ± 23^{e}	Chuhuica (B)	197 ± 32^{abcde}
Kadaknath (I)	288 ± 35^{e}	Yellow Aseel (I)	214 ± 33^{bcde}
Mwanza (T)	311 ± 21^{ef}	Mbeya (T)	251 ± 34^{cdef}
Coast (T)	317 ± 44^{ef}	Moshi (T)	292 ± 31^{cdefg}
Singida (T)	318 ± 26^{ef}	Songea (T)	322 ± 64^{defg}
Fongo-Tongo (C)	330 ± 31^{efg}	Indian Nana- (I)	336 ± 18^{efg}
Arusha (T)	401 ± 71^{efgh}	Dschang (C)	376 ± 21^{fg}
Dschang (C)	440 ± 23^{fgh}	Arusha (T)	402 ± 43^{fg}
Indian Ff- (I)	451 ± 52^{fghi}	Indian Ff- (I)	409 ± 39^{fg}
Balepie (C)	558 ± 28^{ghi}	Bafou (C)	464 ± 48^{g}
Songea (T)	561 ± 124^{ghi}	Fongo-Tongo (C)	467 ± 30^{g}
Cotonou (Be)	635 ± 31^{hi}	Balepie (C)	477 ± 15^{g}
Moshi (T)	656 ± 133^{hi}	Foto (C)	517 ± 61^{gh}
Yellow Aseel (I)	675 ± 64^{i}	Cotonou (Be)	663 ± 58^{h}

$^{a–i}$Means with the same letter in a row shows no significant difference

B, Bolivian breeds; Be, Benin breeds; I, Indian breeds; C, Cameroon breeds; T, Tanzanian breeds

Grouping into ecotypes

In Figure 1, Euclidean distances between the complement titres of the 639 indigenous chickens of the various ecotypes and phenotypes are shown. As can be seen from this dendrogram, the Benin chickens (high CPW and high APW) differ clearly from the Bolivian chickens (low CPW and low APW), whereas ecotypes from Cameroon, Tanzania and lines from India show various combinations of CPW and APW titres.

Figure 2 demonstrates the overall distribution of both CPW and APW titres of all 639 indigenous chickens studied. Most (75%) birds have titres lower than 500 CH_{50} U/ml (CPW and APW). The distribution is clearly skewed to the right. Median titres of the classical pathway for the indigenous avian populations were highest in Benin (600

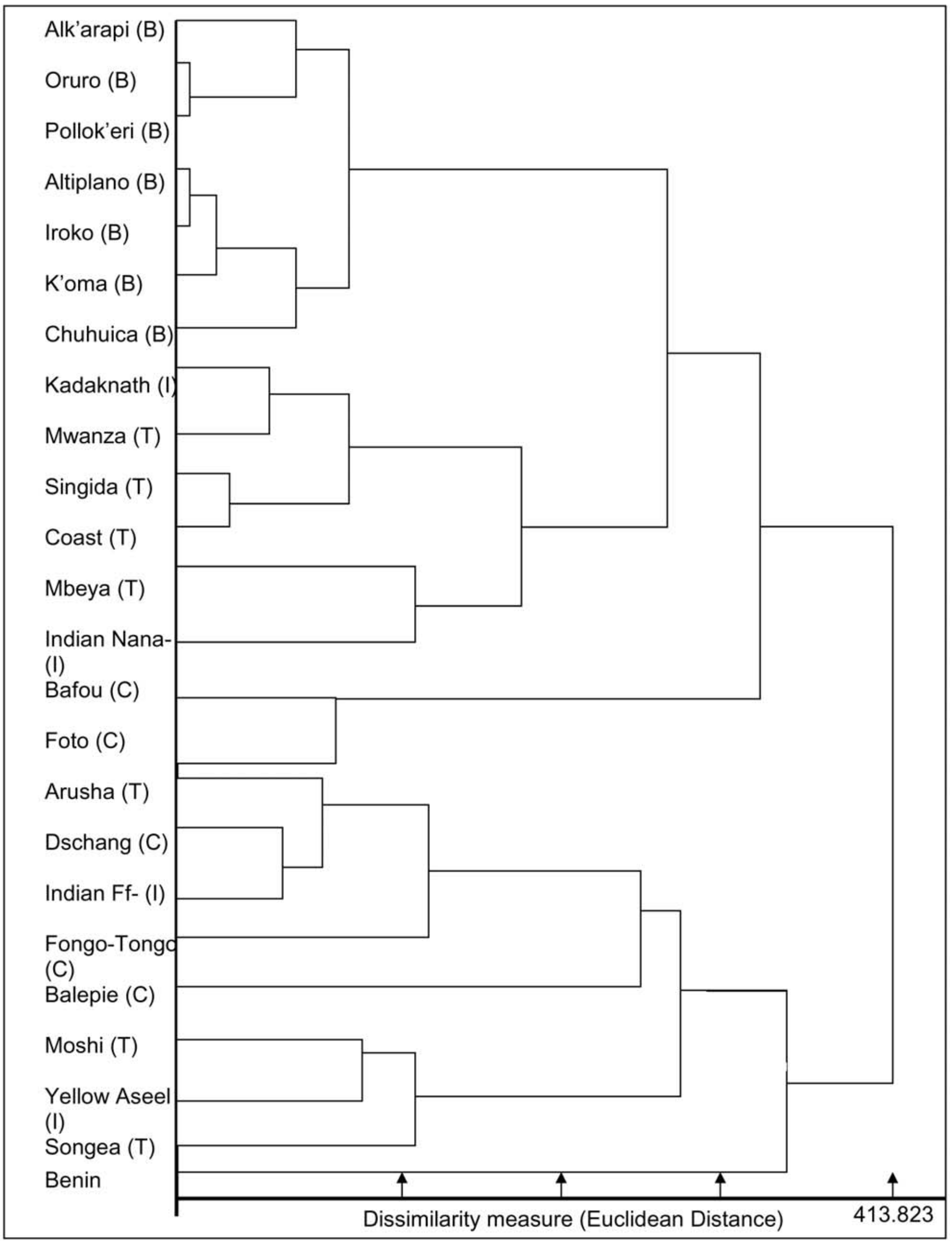

Figure 1. Dendrogram based on the complement titres of 638 indigenous hens. B: Bolivian breeds, I: Indian breeds; C: Cameroon breeds; T: Tanzanian breeds

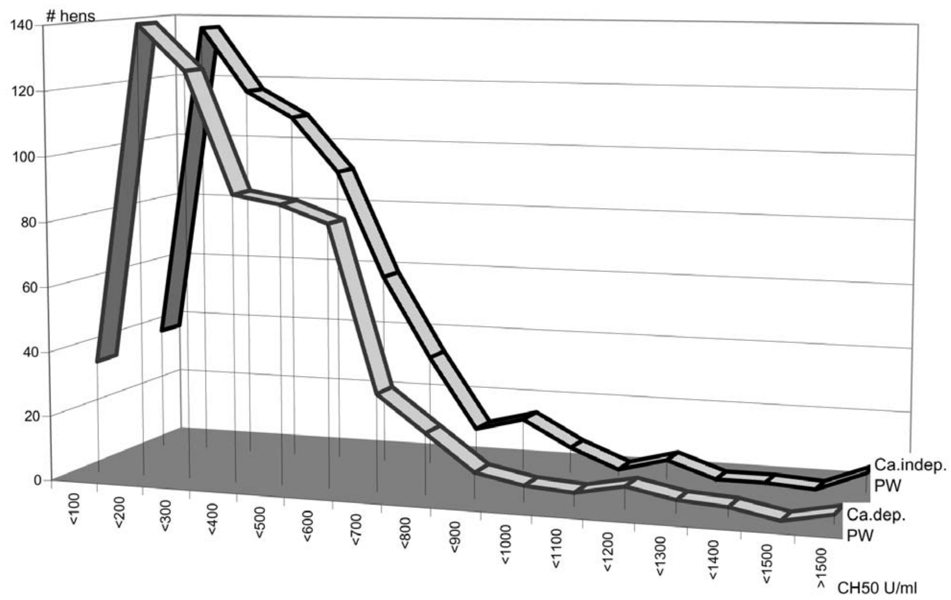

Figure 2. Distribution of Ca-dep.PW and Ca-indep.PW titers of indigenous hens from different regio, ecotypes and countries

CH_{50}U/ml) followed by India (351 CH_{50} U/ml), Cameroon (320 CH_{50} U/ml), Tanzania (286 CH_{50} U/ml) and Bolivia (114 CH_{50} U/ml). The ranking order of the alternative complement pathway titres was Benin (530 CH_{50} U/ml), Cameroon (370 CH_{50} U/ml), India (259 CH_{50} U/ml), Tanzania (209 CH_{50} U/ml) and Bolivia (143 CH_{50} U/ml). Correlations ranging from $r = 0.24$ (India) to $r = 0.29$ (Bolivia) between the Ca-dependent PW and the Ca-independent PW titres were found within the ecotypes of the different countries.

DISCUSSION

Studies on the complement system of the chicken have revealed it as an immunologically important parameter that reacts immediately when it comes into contact with an antigen. Haemolytic complement activity is extremely important in early (innate) host defence. Activated complement neutralizes poultry viruses (Skeeles *et al.*, 1979; Pandit *et al.*, 1997) and parasites (Touray *et al.*, 1994), and results in lysis of bacteria (Patel and Jaiswal, 1994; Nolan *et al.*, 2002). In mammals, the complement system performs various functions. both as an initiator and as an effector system of the innate immune system. In addition, complement components play a role in the initiation and regulation of the specific immune response (Thorbecke *et al.*, 1994). As part of the innate immune barrier, the complement system serves as a buffer between the moment of infection and the time required for the stimulation and triggering of specific cellular and humoral immune responses. In regions where no proper vaccines are available, the (innate) immune system is even more important, owing to constant velogenic disease risks of the birds.

Native populations of chickens are adapted to local conditions and are therefore a valuable genetic resource. This population is a pool of heterogeneous individuals with different phenotypic characteristics (adult body weight and size, body and shank length, comb type, etc.). Characterization of such populations for their immune competence or innate-immune status provides baseline data and information on the immune-mediated adaptation of these birds to local pathogens.

In the current study we found a wide variation in the distribution of complement levels of indigenous chickens. Differences are often more pronounced within ecotypes than between ecotypes. The effect of the genetic background of the various populations on the CPW and APW titres is corroborated by the observation that ecotypes within a geographical location (Cameroon, India, Tanzania) differed in CPW and APW titres. However, the differences in complement titres between Benin (and other African) birds on the one hand and the Bolivian birds on the other suggest that high initial complement levels are a prerequisite for survival under, or as a result of, different environmental pressures.

Several examples can be given. The communities of Foto and Fongo-Tongo in Cameroon are situated about 5 km from each other, and the hens show a large dissimilarity in CPW titres. In the same area, Dschang hens that were bred at the University campus for more than a year (and were thus well protected, sheltered and vaccinated – more than 3 months before sampling to exclude influence of the vaccine on complement levels), demonstrate similar CPW values to chickens from rural Fongo-Tongo. Hens from the northern region of Tanzania (Mwanza and Arusha) have distinct APW status. Regarding Bolivian hens, all ecotypes lived in the same geographical environment of high altitude and consequently were exposed to similar homozygous stress factors of low antigenic pressure and high pathological stress (ascites and cold). This results in birds of a similar phenotype: small and well-feathered. Innate immunity, as measured by haemolytic complement activity, was similar among these birds. The Indian hens could be classified into lines rather than ecotypes. The highest dissimilarity index for complement titres was found in the Indian ecotypes compared with chickens from other countries. We conclude that classifying indigenous hens by ecotypes results in a large diversity of complement titres, which is probably due to a complex of many factors that influence immune status and is therefore not very informative. On the other hand, phenotypic characterization of birds, and implementation of the environment as one of the decisive factors, seems to be a better approach (Msoffe *et al.*, 2001).

The distribution of both CPW and APW was not normal but was skewed to the right. The majority of the hens possess low complement titres, suggesting either low innate immunity or an inactive status of the innate immune system. Nevertheless, these lines have survived for generations in a hostile environment, albeit at great cost of lives each year. However, one may argue that natural selection should result in high primary immune defences. A constant antigenic pressure may then continuously exhaust the avian first-line defence. Differences in the distribution of complement levels in indigenous chickens may reflect different environmental pressures or 'breed' effects or a combination of both. In another study (Parmentier *et al.*, 2004), we described a normal distribution of both CPW and APW titres of 11 commercial European White Leghorn and ISA Brown medium-heavy layers. A normal distribution of complement titres in the commercial lines is not unlikely, since most of these lines are specifically selected and bred for numerous generations. Surprisingly, CPW titres in these commercial lines were also much higher (on average between 580 and 950 CH_{50} U/ml) than in the currently described indigenous chickens. This suggests that, with respect to the CPW titres, either the indigenous hens have initial lower CPW titres or the consumption of CPW complement components in the local chickens is much higher as a result of a more chronic 'microbial' pressure compared to the commercial lines, which are usually kept under supposedly more hygienic conditions. Since we found the highest CPW titres and complement consumption in chickens genetically selected for high antibody responses (Parmentier *et al.*, 2002), a survey on humoral immune responses in indigenous birds might give additional information on relationships between complement and antibody levels (Parmentier *et al.*, in press).

High complement levels (Parmentier *et al.*, 2002; Parmentier *et al.*, 2004) and higher disease resistance (Bacon and Witter, 1992, 1994ab; Hepkema *et al.*, 1993; Pinard *et al.*, 1993; Parmentier *et al.*, 2001) were found in (commercial) layer birds with chicken major histocompatibility haplotypes B2 and B21, and low complement levels (Parmentier *et al.*, 2004) and disease susceptibility (Bacon and Witter, 1992, 1993) in (commercial) layer birds with B15 and B13 haplotypes. Thus, the major proportion of the current indigenous chickens we studied show B13/B15-like levels of complement activity and only a few of the birds have complement levels corresponding to the commercial B2 or B21 birds. Consequently, it is important to study the correlation between the B-haplotypes and complement levels of indigenous village chickens. We determined a large fraction of the Bolivian birds as showing the B15 haplotype, whereas a lack of appropriate binding of BG antigens from Indian breeds by available alloantisera indicated a potentially large pool of unknown BG haplotypes (Baelmans *et al.*, in press). Such information might add to the selection of immune-competent lines as well as revealing the biological relationship between the innate and specific immune systems in field chickens. In addition, the lack in the current study of a (cor)relation between CPW and APW titres in most populations suggests that selection of indigenous breeds for enhanced immune-mediated disease resistance requires further study on the physiological relationship between CPW and APW and the effects of genetic background and/or environment on CPW or APW consumption.

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Différents taux de complément hémolytique dans le sérum chez les poulets natifs du Bénin, de la Bolivie, du Cameroun, de l'Inde et de la Tanzanie

Résumé – Les titres de relais de compléments classiques (CPW) et alternatifs (APW) ont été mesurés dans des populations de poulets locaux cliniquement en bonne santé (écotypes) venant d'Afrique (Bénin, n = 78; Cameroun, n = 299; Tanzanie, n = 101), d'Asie (Inde, n = 96) et d'Amérique du Sud (Bolivie, n = 64). Une grande variation a été découverte dans les taux de complément hémolytique entre les divers écotypes. Les distributions des titres de compléments classiques et hémolytiques ne se sont pas révélées normales mais ont été biaisées vers la droite. Des différences dans les compléments ont été mises à jour à la fois parmi et entre les écotypes. Et qui plus est, les titres de CPW des poulets natifs ont été inférieurs à ceux déterminés chez les poules pondeuses commerciales. Ceci suggère que les taux de compléments des poulets natifs ne sont pas uniquement dépendants de la pression antigénique locale mais qu'ils dépendent également des antécédents génétiques et de la gestion des animaux. La relation entre les taux de compléments, le complexe MHC(B) des poulets, la pression antigénique environnementale et la survie des poulets locaux pilleurs de poubelles sont discutées.

Diferentes niveles de complemento hemolítico en el suero en pollos indígenas de Benin, Bolivia, Camerún, India y Tanzania

Resumen – Se midieron títulos de complemento por rutas clásica (CPW, en inglés) y alternativa (APW) en poblaciones de pollos locales clínicamente sanos (ecotipos) de Africa (Benin, n = 78; Camerún, n = 299; Tanzania, n = 101), Asia (India, n = 96) y Sudamérica (Bolivia, n = 64). Se encontró una gran variación en los niveles de complemento hemolítico entre los distintos ecotipos. Las distribuciones de los títulos de complemento por vía clásica y alternativa no resultaron normales sino desviadas a la derecha. Se encontraron diferencias en cuanto al complemento dentro y entre los ecotipos. Además, los títulos o cantidades de complemento por vía clásica de los pollos indígenas eran más bajos que la de aquellos determinados en pollos de puesta comerciales. Esto sugiere que los niveles de complemento en las gallinas indígenas no sólo dependen de la presión del antígeno local sino que también puede depender del historial genético y de la agricultura. En este artículo se discuten las relaciones entre los niveles de complemento, el complejo principal de histocompatibilidad (B) del pollo o MHC (B), la presión antigénica medioambiental, y la supervivencia de los pollos carroñeros locales.