Emerging threat of *Eimeria* operational taxonomic units (OTUs) on poultry production

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Abstract

Coccidiosis is an antagonistic poultry disease which negatively impacts animal welfare and productivity. The disease is caused by an obligate, intracellular protozoon known as *Eimeria*. Several *Eimeria* species known to infect chickens have been well documented. However, recent studies have elucidated the emergence of three novel genetic variants or operational taxonomic units (OTUs). The discovery of OTUx, OTUy and OTUz complicates the identification and diagnosis of coccidiosis. OTUs are clusters of unknown or uncultivated organisms that are grouped according to a similarity in DNA sequence to a set of specific gene markers. OTUs have been reported in the Earth's Southern Hemisphere, including Australia, Venezuela, India, Zambia, Uganda, Tanzania, China and Ghana. Elucidating their impact on the poultry industry is fundamental in preventing anticoccidial resistance and to access the potential of OTUs as vaccine candidates to provide cross-protection against similar *Eimeria* species. The identification of OTUs further decreases the risk of false negative coccidial diagnosis. Therefore, this article reviews the importance and risk imposed by OTUs, coupled with their prevalence and geographical distribution in chickens globally.

Introduction

The poultry industry is a major driving force of food security and the economy worldwide (Mottet and Tempio, 2017). In 2025, global poultry production is set to increase to 131 255 metric tons (Poultry Hub, 2019). Mohammed and Sunday (2015) reported a production of 1.125 million different birds of poultry in Africa alone. Chickens, a common source of poultry, are fast becoming the most consumed meat throughout the world (Boulton *et al.*, 2018). Approximately 60 billion chickens per annum are produced globally, yielding 90 million tons of meat and over a trillion eggs (Clark *et al.*, 2017). Chikens' protein content, short generation interval and global availability make them an economically significant poultry species (Venkatas *et al.*, 2019). However, the productivity and welfare of chickens are compromised by several diseases, including coccidiosis (Adenaike *et al.*, 2018; Hamid *et al.*, 2018; Macdonald *et al.*, 2019; Venkatas and Adeleke, 2019).

Coccidiosis is a parasitic disease caused by a protozoan known as *Eimeria* (Brown-Jordan *et al.*, 2018). Guven *et al.* (2013) reported the prevalence of coccidiosis infection to vary between 10% and 90% in industrial chicken farms worldwide. The high reproductive potential and short, complex life-cycle of *Eimeria* often leads to severe disease outbreaks in both large commercial farms and small backyard flocks. Sporulated oocysts of *Eimeria* invade and damage the epithelial lining of the infected animal's intestinal cells (Cheng *et al.*, 2018). This in turn, reduces the absorption of nutrients across the wall of the gut, resulting in poor growth and performance, as well as, leaving the infected chickens susceptible to secondary diseases (Adewole *et al.*, 2017; da Costa *et al.*, 2017). In addition to compromising the health of chickens, the disease has incurred a large economic loss exceeding \$3 billion. Hence, developing a means of controlling the disease is fundamental (Cheng *et al.*, 2018).

There are seven known *Eimeria* species, each of which differs significantly by both morphological and physiological means. The identification of each species present within an infection is vital to develop species-specific treatment, as all *Eimeria* species may not be susceptible to standard treatment (Guven *et al.*, 2013; Brown-Jordan *et al.*, 2018; Fatoba and Adeleke, 2018; Sharma *et al.*, 2018). The identification of *Eimeria* species is not only required to diagnose and control the disease but is also required for population genetics, biology and epidemiological studies (Huang *et al.*, 2017).

Recent studies have reported the identification of three new genetic variants of *Eimeria*, known as operational taxonomic units (OTUs), which further complicate the identification and diagnosis of coccidiosis (Jatau *et al.*, 2016). Three chicken OTUs, namely OTUx, OTUy and OTUz, were detected in Australia (Godwin and Morgan, 2015), Nigeria (Jatau *et al.*, 2016) and several other geographical regions below the 33° N line of latitude (Fornace *et al.*, 2013; Clark *et al.*, 2016). However, their rapid spread north is of great concern as they limit the effectiveness of current anticoccidial vaccines (Fatoba and Adeleke, 2018). There is also a dearth of information regarding the pathology, risk imposed, occurrence and drivers of OTUs worldwide. Therefore, this article aims to provide a review of the risk imposed by OTUs on poultry industry, their prevalence and geographical distribution

in order to determine their potential as anticoccidial vaccine candidates and to reduce the incidence of false negative coccidial diagnosis.

Impact and diagnosis of poultry coccidiosis

Mild or sub-clinical *Eimeria* infections are often difficult to diagnose as infected birds do not display any obvious symptoms (Shahraki *et al.*, 2018). Clinical *Eimeria* infections take a great toll on infected birds, resulting in a sudden drop in feed intake and a reduction in essential vitamin and mineral absorption. Symptoms include bloody diarrhoea, fatigue, dehydration and eventually death (Eke *et al.*, 2016). Natural coccidial infections in chickens are caused by a single *Eimeria* species or a combination of different *Eimeria* species (Guven *et al.*, 2013; Gadelhaq *et al.*, 2015; Giles *et al.*, 2019). The severity or level of pathogenicity observed in an infection depends on the species of *Eimeria* causing the infection (Brown-Jordan *et al.*, 2018). *E. mitis* and *E. praecox* (low pathogenicity) and *E. acervulina* and *E. maxima* (moderate pathogenicity) induce a mild coccidial infection, whereas *E. brunetti*, *E. necatrix* and *E. tenella* (high pathogenicity) bring about internal haemorrhaging, predisposing the birds to necrotic enteritis and, in rare instances, lead to death (Barbour *et al.*, 2015; Huang *et al.*, 2018; Macdonald *et al.*, 2019).

Understanding the pathogenicity, genetic diversity, prevalence and global distribution of these parasites is vital to develop robust, effective and long-term control measures (Brown-Jordan *et al.*, 2018). Morphological (oocyst shape and size) and pathological (lesion site) analyses were used to successfully diagnose coccidial infections. However, the analysis only partially identified each *Eimeria* species (Arafat and Abbas, 2018; Yunus *et al.*, 2019). These detection methods require trained experts, and are labour-intensive and time-consuming (Ogedengbe *et al.*, 2011). Molecular, biochemical and immunological techniques have been developed to overcome these limitations (Boulton *et al.*, 2018). According to Clark *et al.* (2017), DNA-based approaches, such as assessment of the iso-enzyme patterns of oocysts, rRNA and rDNA probes and species-specific PCR approaches have been established. DNA diagnostic approaches rely on the comparison of orthologous sequences to identify the species of a genus (Sharma *et al.*, 2018). Tang *et al.* (2018*a*) postulated that *Eimeria* species have been effectively and accurately identified using the 18S rDNA region or ITS-1 and ITS-2 sequences. Recent studies have reported the identification of three new genetic variants of *Eimeria*, OTUs, which further complicate the identification, diagnosis and treatment of coccidiosis (Jatau *et al.*, 2016).

Eimeria operational taxonomic units (OTUs) in chickens

Advances in DNA sequencing technology enabled researchers to unveil and study microorganism communities at far greater depths in the past decade (Huang *et al.*, 2018). These studies involve the amplification and sequencing of gene markers, such as the internal transcribed spacer regions (ITS-1/2) and 16S small ribosomal subunit RNA (rRNA). Amplified gene markers are then aligned to known sequences in genetic databases for identification (Hinsu *et al.*, 2018; Stenzel *et al.*, 2018). To date, a large number of novel organisms has been discovered, including novel genetic variants of *Eimeria*, OTUx, OTUy and OTUz (Jatau *et al.*, 2016). The term 'operational taxonomic unit' was first coined by Sneath and Sokal (1962). OTUs were initially regarded as a group of individuals clustered together by similarity and equivalence to but not necessarily in line with evolutionary taxonomy (Callahan *et al.*, 2017). However, studies describe OTUs as a pragmatic proxy for different taxonomic levels of microbial species in the absence of traditional systems of biological

classification (Vermeulen *et al.*, 2016; Huang *et al.*, 2018). Therefore, OTUs are clusters of unknown or uncultivated organisms that are grouped according to a similarity in DNA sequence to a specific taxonomic gene marker (Callahan *et al.*, 2017).

Three *Eimeria* OTUs in chickens, namely OTUx, OTUy and OTUz have been reported to date (Cantacessi *et al.*, 2008; Fornace *et al.*, 2013; Godwin and Morgan, 2015; Clark *et al.*, 2016). Clark *et al.* (2017) attributed the emergence of OTUs to be a consequence of geographical isolation, genetic drift and mutations. According to Wang *et al.* (2019), the distribution OTU-infected broiler chickens worldwide disseminated novel OTUs. *Eimeria* OTU infections cannot be treated or controlled with traditional anticoccidial prophylaxis developed for known *Eimeria* species (Huang *et al.*, 2018; Sakkas *et al.*, 2018). Hence, it is fundamental to access the potential of OTUs as candidates for anticoccidial vaccines by addressing the impact of OTUs on the global poultry industry (Vermeulen *et al.*, 2016).

Impact of *Eimeria* OTUs in poultry production

The economic impact of *Eimeria* OTUs is yet to be accurately established, as the presence of the genetic variants were only recently reported (Morgan and Godwin, 2017). Hence, in addition to a decrease in production, additional funds are now required for the development of new strategies to identify OTUs, coupled with the development of novel OTU antigen vaccines (Vermeulen *et al.*, 2016; Boulton *et al.*, 2018). OTUx resulted in a gross margin of $0.1 per broiler bird and $3.4 per layer bird per annum in Tanzania; $0.1 per broiler bird and $−0.6 per layer bird per annum in Zambia; $1.3 per broiler bird and $−0.4 per layer bird per annum in Ghana. OTUz generated a gross margin of $−3.6 per broiler bird and $−2.6 per layer bird per annum in Tanzania; $−1.9 per layer bird per annum in Zambia; and $−0.3 per broiler bird and $−2 per layer bird per annum in Ghana. However, these figures included additional expenses of feed and daily operational costs (Fornace *et al.*, 2013).

Although these genetic variants have only been noted in the southern hemisphere, human movement and trade add to the risk of the spread of OTUs (Fornace *et al.*, 2013; Clark *et al.*, 2016; Jatau *et al.*, 2016; Morgan and Godwin, 2017). This in turn confers significant consequences for the development and application of novel anticoccidial vaccines, as OTU variants may evade the immune protection conferred by vaccines (Clark *et al.*, 2017; Hinsu *et al.*, 2018). Godwin and Morgan (2015) confirmed the lack of or little protection of developed vaccines against these genetic variants. The authors further stated the dearth of information regarding the biology and exact impact of OTUs on poultry production in terms of how each OTU negatively affects the birds. For example, Paracox 8 (×10) and HuveGuard MMAT anticoccidial vaccines effectively retarded infections with known *Eimeria* species. However, the vaccine showed little to no effect on coccidial infections with OTUx or OTUz. Jatau *et al.* (2016) reported similar results, whereby Eimeriavax® 4 M which effectively treats against *E. tenella*, *E. necatrix*, *E. maxima*, and *E. acervulina* could not prevent colonization by the OTU genetic variants (Chang *et al.*, 2015). This is of growing concern especially in countries where antibiotics have been phased out, resulting in vaccines being utilized as the sole anticoccidial treatment measure.

The impact of OTUs has been undermined due to a lack of mortality reports and indiscrete lesions. However, OTUx was reported to be moderately pathogenic, resulting in malabsorption. On the other hand, OTUy and OTUz were said to be highly and very highly pathogenic, respectively, resulting in haemorrhage (Clark *et al.*, 2017). The relationship and distinctiveness between OTUs and known *Eimeria* species affecting chickens aid in
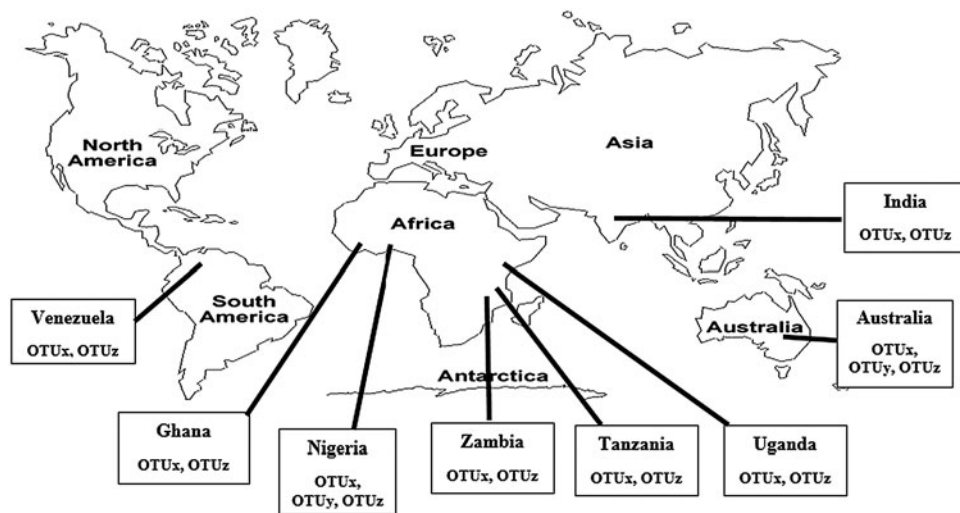
Fig. 1. The geographical distribution of reported Eimeria genetic variants, OTUx OYUy and OTUz throughout the world (Fornace *et al*., 2013; Clark *et al*., 2016; Jatau *et al*., 2016; Morgan and Godwin, 2017).

uncovering the genetic variability of the variants (Godwin and Morgan, 2015; Jatau *et al*., 2016; Hinsu *et al*., 2018).

Eimeria OTUS: divergent strains or distinct species?

One thousand eight hundred *Eimeria* species have been noted in a vast range of hosts, including domestic pets (cats and dogs), poultry (chicken, turkey, duck) and ruminants (cattle, goats and sheep) (Chapman, 2014; Blake, 2015; Silva *et al*., 2017). However, only seven pathogenic *Eimeria* species have been noted in chickens, including *E. praecox*, *E. tenella*, *E. necatrix*, *E. mitis*, *E. maxima*, *E. brunetti* and *E. acervulina* (Clark *et al*., 2016). Each of these species differ in prevalence, location of infection, pathogenicity and immunology depending on the magnitude of the infection (Barbour *et al*., 2015; Huang *et al*., 2017; Brown-Jordan *et al*., 2018).

The bayesian species delimitation statistical analysis tool using a Markov chain Monte Carlo (reverse jump) method was utilized to access the relationships between OTUs and known *Eimeria* species (Hinsu *et al*., 2018). Clark *et al*. (2017) and Morgan and Godwin (2017) described the similarity between the OTUx sequence and ITS2 sequence of *E. maxima*, whereby OTUx clustered alongside *E. maxima* in a phylogenetic Tamrua-Nei model. The authors also detected the close cluster of OTUy and *E. brunetti* sequences, whereas OTUz sequences formed a distinct clade by itself. Cantacessi *et al*. (2008) and Fornace *et al*. (2013) reported similar results. However, Fornace *et al*. (2013) defined OTUz as a genetic descendant of *E. necatrix* and *E. tenella*. The similarity detected between OTUz and known *Eimeria* species in chickens were further complicated when Godwin and Morgan (2015) observed significant similarity between OTUz and *E. mitis*.

The short distance between OTUx and *E. maxima* signifies a close relationship, indicating that the genetic variants are sister species. However, the genetic variant differs significantly from *E. maxima* in oocyst shape and size, pre-patent period and the site of infection. Similar differences were observed between OTUy and *E. brunetti* (Godwin and Morgan, 2015; Jatau *et al*., 2016; Hinsu *et al*., 2018). Clark *et al*. (2017) suggested that the great divergence between OTUz and respective *Eimeria* species indicate that OTUz should be regarded as a novel species. While each of the OTUs was partitioned separately and were confidently taxonomically assigned, an analysis of additional loci with greater stability is required (Hinsu *et al*., 2018). Characterization and alignment of OTUs to global *Eimeria* isolates aid in the creation of genetic markers used to identify the genetic variants

(Morgan and Godwin, 2017). However, to determine whether OTUs have recently diverged from closely related *Eimeria* species the prevalence, characterization and geographical distribution of these OTUs need to be accessed (de Macedo *et al*., 2019).

Prevalence and geographical distribution of Eimeria OTUs

The occurrence and prevalence of *Eimeria* species depend on both intrinsic (host age and class) and extrinsic (farm and flock size; seasonal variation and environmental factors, coupled with management strategies) factors (Kadykalo *et al*., 2017). Godwin and Morgan (2015) reported OTUy to be the most widespread and abundant genetic variant in Australia with more than 45% of flocks infected, whereas OTUz was the least common (8%) and OTUx (13%) was only moderately observed. The authors further illustrated the prevalence of OTUs to be equivalent to the prevalence of common *Eimeria* species; *E. maxima, E. tenella* and *E. brunetti* in commercial flocks globally. Hinsu *et al*. (2018) reported similar results in India with OTUy being the most dominant genetic variant.

OTUx and OTUy were initially discovered in Victoria, Australia, in two vaccinated flocks of broiler chickens (Morris *et al*., 2007). Hinsu *et al*. (2018) confirmed these results and concluded that OTU variants were more common in the Northern region of Australia with 188 OTUs identified out of 592 samples. However, all three OTUs (OTUx; OTUy and OTUz) were observed at an average occurrence of 33% in both commercial and backyard farms throughout Australia (Cantacessi *et al*., 2008; Godwin and Morgan, 2015; Morgan and Godwin, 2017). North Western Nigeria had a similar prevalence, with all three OTUs detected (Jatau *et al*., 2016). The presence of only two variants, OTUx and OTUz, were noted in commercial farms throughout Zambia, Tanzanian and Ghana (Fornace *et al*., 2013). Clark *et al*. (2016) observed similar results in Venezuela, India, Zambia, Uganda, Tanzanian and Ghana. The authors further reported a dispersion of the two cryptic variants in quantities comparable to *E. necatrix* and *E. maxima* throughout South America, Sub-Saharan Africa and Southern Asia

Hinsu *et al*. (2018) reported similar results, detecting low levels of OTUy in India using Next Generation Sequencing (NGS) technology, thus providing the most northern occurrence of OTUs reported to date. Tang *et al*. (2018*b*) recently observed additional, unidentifiable OTUs in chickens immunized with transgenic *Eimeria* in China. The occurrence of OTUs is only observed in

the Southern Hemisphere (30°N latitude) (Fig. 1) and is yet to be discovered in South Africa, Egypt, USA and Europe (Clark *et al.*, 2017). The spread of OTUs north of the 30°N line of latitude is of growing concern. Hence, identifying the factors driving the global distribution of OTUs is fundamental for coccidial control.

Factors influencing the polarized distribution of *Eimeria* OTUs

The elucidation of factors driving the polarized distribution of OTUs globally was initially brought into question by Clark *et al.* (2016). The authors suggest the polarized distribution of OTUs may result from the discovery of novel trade routes for the distribution of goods, including commercial chicken broilers. Commercial chicken genotypes have recently been introduced to much of the southern hemisphere. These genotypes interbreed with indigenous chicken breeds, introducing OTU genotypes into chicken populations (Suganthi, 2014; Morgan and Godwin, 2017). However, the lack of persistence and nature of infection within a host make transmission by this means highly unlikely (Jatau *et al.*, 2016). Additionally, commercial poultry breeds were historically dispersed north to south, as determined by ancient mitochondrial DNA signatures (Storey *et al.*, 2012). OTUs are also said to migrate *via* transmission machinery; equipment and farm workers; genetic drift *via* geographical isolation and switching hosts from other galliforms with a regional distribution (Clark *et al.*, 2016). Novel trade routes increase the likelihood of the parasite population mixing (Ogedengbe *et al.*, 2011).

Conversely, Hinsu *et al.* (2018) reported a significant similarity in OTUy genotypes detected in indigenous and commercial chicken breeds in India. This indicated that the polarized global distribution of OTUs is highly unlikely to be a consequence of host genotype specificity. Clark *et al.* (2017) and Prakashbabu *et al.* (2017) reported that OTUs are more dependent on geographical location as compared to the seven-known chicken *Eimeria* species. However, geographical location alone serves as an inadequate explanation for the polarized distribution of OTUs. The origin of OTUs is yet to be discovered due to a lack of historical samples. Therefore, additional studies should be carried out to determine the geographical origin of OTUs (Fornace *et al.*, 2013; de Macedo *et al.* 2019). However, OTUs could be distinct species of *Eimeria*, which diverged a long time ago, together with the seven known *Eimeria* species as opposed to diverging recently from closely related species. Thus, OTUs may be ubiquitous, but due to limited methods of detection and discrimination, may not be detected. All gene markers used to detect specific *Eimeria* species, i.e. ITS-1, ITS-2, 18s ribosomal and SCAR markers, are located within a single locus. Genome-wide association studies (GWAS) should be carried out on OTU variants to identify more effective gene markers for identification.

Conclusion

The elucidation of *Eimeria* genetic variants, OTUx, OTUy and OTUz, has resulted in great controversy over the last decade by thwarting the identification and diagnosis of coccidiosis. Understanding their impact on the economy and poultry industry is necessary to prevent further anticoccidial resistance and access the anticoccidial vaccine potential of OTUs. Although there is a dearth of information regarding the biology and exact impact of OTUs on poultry production, OTUx was reported to be moderately pathogenic, resulting in malabsorption. OTUy and OTUz were said to be highly and very highly pathogenic, in the order listed. The relationship and distinctiveness between OTUs and known *Eimeria* species affecting chickens may help to unravel the biology behind these genetic variants. The effectiveness of current vaccines is also limited by the prevalence and geographical distribution of OTUs, as the prevalence of OTUs varies in different geographical regions. To date, the occurrence of OTUs was only noted in the Earth's Southern Hemisphere. However, migration of OTUs to the rest of the world may be inevitable. However, the detection of OTUs in Northern regions of the world is limited by genetic markers occurring within a single locus. Characterization and GWAS should be carried out on OTU variants to identify more effective gene markers for identification. Additional research into the biological aspect of OTUs and their potential as anticoccidial vaccine candidates to provide cross-protection against their closest relatives is required.

Conflict of interest. The authors declare that they have no conflict of interest.

Ethical standards. Not applicable.

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