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Research Article

Serological Evidence of *Henipavirus* among Horses and Pigs in Zaria and Environs in Kaduna State, Nigeria

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Henipavirus is an emerging, zoonotic, and lethal RNA virus comprising *Hendra virus* (*HeV*) and *Nipah virus* (*NiV*), to which fruit bats are reservoir. Husbandry practices in Nigeria allow close contact between bat reservoir and animals susceptible to *Henipavirus*. This cross-sectional survey investigated antibodies reactive to *Henipavirus* sG antigen and associated risk factors in horses and pigs in Zaria, Nigeria. Using convenience sampling, 510 sera from horses ($n = 200$) and pigs ($n = 310$) were screened by an indirect *Henipavirus* enzyme-linked immunosorbent assay (ELISA) (CSIRO, Australia). Structured questionnaires were employed with questions on the demographics and management of the animals. Data were analysed using SPSS-17. 5. Seroprevalence was higher for horses managed intensively (21.1%); used for sports (25.5%); watered with pipe borne water (17.9%); fed commercial feed (22.3%); and fed in the pen (17.6%). Seroprevalence was higher for pigs managed intensively (58.1%); imported (69.5%); watered with pipe-borne water (31.3%); fed commercial feed (57.4%); fed in the pen (23.4%), and fed with feed prestored in a feed house (49.5%). Horses <5 years and pigs <6 months had higher seroprevalences of 18.1% and 21.3%, while the female horses and pigs had seroprevalences of 19.8% and 22.8%, respectively. Exotic horses and pigs revealed 25.5% and 55% and horses in Igabi and pigs in Giwa revealed 24.7% and 70.2% seroprevalence, respectively ($P < 0.05$). There is a suggestive evidence of *Henipavirus* in horses and pigs in Zaria, Nigeria, with a huge public health implication. Local and exotic pigs and horses, pigs in Zaria and Sabon-Gari, and horses in Zaria, Sabon-Gari, and Kaduna North are associated with the seroprevalence of henipaviruses.

1. Introduction

In our world today, diseases are emerging and reemerging, among which is *Henipavirus*, a genus comprising two emerging zoonotic and highly lethal viruses (*Hendra virus* (*HeV*) and *Nipah virus* (*NiV*)) within the familyParamyxoviridae. Pteropid bats are the main reservoir for these closely related viruses causing highly fatal diseases in animals and human [1–4]. Bat paramyxoviruses cause versatile impact on livestock and human health leading to zoonotic infections and epizootics reportable to the World Organization for Animal Health (OIE) [5–8].

In 1994, a disease outbreak that affected thoroughbred race horses and human in Brisbane suburb of Hendra, Queensland, was the first incidence of *HeV* [9]. There had been previous occurrence of these viruses in horses and also in a single human case (at Mackay, further north of Queensland) mistaken for equine morbillivirus [10, 11]; extensive survey revealed that Australian horses were not the likely source of infection [9, 12]. *NiV* first emerged in 1998-99 in Malaysia and Singapore where it caused acute febrile encephalitis in humans [13]. This major outbreak resulted in 265 human cases that claimed 105 lives in Malaysia [14]. In Singapore, it resulted in 11 cases and a death among abattoir workers who handled carcases of pigs imported from affected regions of Malaysia [15, 16].

Transmission cycles of *HeV* and *NiV* to man are unalike; *HeV* is first transmitted from bats to horses and then to man

while *NiV* is first transmitted from bats to pigs then to man and also directly from bats to man as well as man to man [17–20].

HeV and *NiV* are in the list of most deadly zoonotic viruses and have resulted in deaths or euthanasia of over 80 horses and four out of the seven humans known to be infected with *HeV* also died. There have been spill-over and recurrent outbreaks [16].

There is evidence of *HeV* and *NiV* in African bats, with other animals and birds holding switch predominance [7]. There is also evidence of anti-*Henipavirus* antibodies in West Africa [21], Annobón island in the Gulf of Guinea [22], and Cameroon. However, there are no relevant data available on *Henipavirus* in Nigeria.

Husbandry practices in developing countries allow close contact between bats and animals susceptible to *Henipavirus* as horses are tethered under trees overnight during recreation and polo. The practices in pig farming especially in villages allow close contact between bats and pigs. Deforestation makes bats leave their niche to urban settlements, bringing them in close contact with man, horses, and pigs. These pose a great risk to livestock farmers, veterinarians, and technicians performing their professional duties. The aim of the study was to detect the presence of *Henipavirus* in horses and pigs and to assess the risk factors for exposure to *Henipavirus* among horses and pigs in Zaria and its environs in northern Nigeria.

2. Materials and Methods

2.1. Sampling. Employing a nonrandom sampling technique, samples were collected with permission granted by local authorities (village/ward head(s)) and animal owners. Horses were sampled from Zaria, Sabon-Gari, Igabi, and Kaduna North while pigs were sampled from Zaria, Sabon-Gari, and Giwa. Following efficient restraint, 5 mls of blood was aseptically collected from 310 pigs and 200 horses by anterior vena cava and jugular venepuncture, respectively. Blood samples collected in the field were transported in cool man box to the laboratory and clear sera from settled blood were harvested while unsettled blood was centrifuged at 3000 rpm for 5 minutes before harvesting the sera. All sera were stored at −20°C until needed for further analyses.

2.2. Henipavirus Antibody Indirect Enzyme-Linked Immunosorbent Assay (HeV sG iELISA). The ELISA used 50 μL/well of *Henipavirus* sG antigen (1 : 3000) diluted in 0.05 M carbonate-bicarbonate ELISA Coating Buffer (pH 9.6) into a 96-well NUNC Maxisorp ELISA plate after which the plates were left stationary overnight at 4°C. The plates were washed three times with Phosphate Buffered Saline Tween (PBST) and blocked for 30 minutes with 2% skimmed milk/Phosphate Buffered Saline Tween at 37°C. Test and control sera were diluted 1 : 100 in 2% skimmed milk/PBS-T dilution buffer and 50 μl was added to the plate and shaken for 1 hr at 37°C. Bound antibody was detected by using Protein A/G-Horse Radish Peroxidase conjugate (Pierce, Rockford, USA) and 3,3′,5,5′,-tetramethylbenzidine dihydrochloride (TMB) substrate (Sigma-Aldrich Pty. Ltd., Australia). Plates were read for absorbance using ELISA reader at an optical density of 450 nm.

2.3. Questionnaire Survey. Data on the age, sex, breed, source of animal, source of feed and water, location, management system, and presence or absence of bat in environment of all animal sampled were recorded by observation and first-hand information from the animal owner/handler.

2.4. Statistical Analysis. Data generated were analysed using the Statistical Packages for Social Sciences (SPSS) Version 17.0. Chi square test was used where appropriate to test for association of variables (age, sex, breed, source, etc.) obtained from the questionnaires. Values of $P < 0.05$ were considered significant.

3. Results

This study identified the occurrence of *Henipavirus* antibodies in pigs and horses sampled in Zaria and environs. Out of the 510 samples, 310 pigs and 200 horses, species-specific prevalence was 20% and 15.5% for pigs and horses, respectively.

3.1. Pig. A total of 197 and 113 pigs in this study fell within the age group <6 months and ≥6 months, respectively (Table 2). Age group specific seroprevalence of 67.7% and 32.3% was obtained for pigs <6 months and ≥6 months, respectively (Table 2). The sex specific seroprevalence was 17.6% and 22.8% for males and females, respectively (Table 2). The breed of pigs included local ($n = 216$), exotic ($n = 60$), and cross breeds ($n = 34$) with antibody prevalence of 11.5%, 55%, and 11.7%, respectively. A statistically significant association existed between the breed of pigs and the seroprevalence ($P < 0.05$) (Table 2).

Thirty-two (69.5%) of the 46 imported pigs were seropositive while 30 (11.3%) of the 264 locally sourced pigs were seropositive. The difference in the seroprevalence was statistically significant ($P < 0.05$) (Table 1). Out of the study population, 155 pigs were sampled from Sabon-Gari, 108 from Zaria, and 47 from Giwa local government areas. A seroprevalence of 70.2%, 13.8%, and 9% was recorded for Giwa, Zaria, and Sabon-Gari, respectively. There was statistically significant difference in seroprevalence by location of sampling (Table 1). Two hundred and eight of the pigs that were watered with well water had 14.4% seroprevalence while 102 watered with pipe-borne water had 31.3% seroprevalence. There was a statistically significant difference between prevalence of *HeV* antibodies ($P < 0.05$) and source of water (Table 1). A total of 256 pigs were fed homemade feed and 54 were fed commercial feed. The feed type prevalence obtained was 12.1% and 57.4% for homemade and commercial feed, respectively. There was a statistically significant difference between prevalence and feed types ($P < 0.05$) (Table 1). A prevalence of 15.2% and 23.4% was recorded for animals fed in the open and animals fed in the pen, respectively. Only ninety-one of the pigs sampled had their feed stored in a feed house before being fed to them; 48 pigs were fed prestored feed in the open

Table 1: Risk factors associated with the prevalence of *Henipavirus* in pigs in Zaria and environs.

Risk factor	Number	Number of seropositive animals (%)	Chi, df	*P* value	Odds ratio	95% CI
Management system						
Intensive	55	32 (58.1)	60.92, 1	<0.0001	10.43	5.41–20.14
Semi-intensive	255	30 (11.7)				
Source of animal						
Imported	46	32 (69.5)	82.94, 1	<0.0001	17.83	8.56–37.25
Locally sourced	264	30 (11.3)				
Source of water						
Well	208	30 (14.4)	12.29, 1	0.0005	0.37	0.21–0.65
Pipe-borne	102	32 (31.3)				
Feed type						
Homemade	256	31 (12.1)	57.19, 1	<0.0001	0.10	0.05–0.20
Commercial	54	31 (57.4)				
Feeding method						
In the open	131	20 (15.2)	3.176, 1	0.0747	0.59	0.33–1.06
In the pen	179	42 (23.4)				
Feed storage						
No storage	171	11 (6.4)	70.69, 2	<0.0001	Ref.	
Open	48	6 (12.5)			0.48	0.17–1.38
Feed house	91	45 (49.5)			0.07	0.03–0.15
Bat presence						
No	128	42 (32.8)	22.4, 1	<0.0001	0.253	0.140–0.458
Yes	182	20 (10.9)				
Total	310	62 (20)				

Table 2: Seroprevalence of antibodies reactive to *Henipavirus* sG antigen in pigs sampled in Zaria and environs based on age, sex, breed, and location.

Variables	Number (%)	Number of seropositive animals (%)	χ^2	*P* value	Odds ratio	95% CI
Age						
<6 months	197	42 (21.3)	0.588	0.443	1.26	0.70–2.28
≥6 months	113	20 (17.6)				
Sex						
Male	170	30 (17.6)	1.303, 1	0.2538	0.72	0.41–1.26
Female	140	32 (22.8)				
Breed						
Local	216	25 (11.5)			0.11	0.06–0.21
Exotic	60	33 (55)	56.96	<0.0001	Ref.	
Cross	34	4 (11.7)			9.17	2.87–29.3
Location						
Zaria	108	15 (13.8)	88.24, 2	<0.0001	0.62	0.28–1.34
Sabon-Gari	155	14 (9)			Ref	
Giwa	47	33 (70.2)			0.04	0.02–0.10
Total	310	62 (20)				

while 171 had no storage plan. The seroprevalence of 6.4%, 12.5%, and 49.5% was obtained from farm without storage, those who stored feed in the open, and those with a feed house, respectively. This difference between prevalence and feed storage was statistically significant ($P < 0.05$) (Table 1). Thirty-two samples (58.1%) from institutional farms managed intensively and 30 samples (11.7%) from backyard farms managed semi-intensively had antibodies to *Henipavirus* antigen. There was statistically significant difference between seroprevalence and farm type/management practice ($P < 0.05$) (Table 1). Only 10.9% of seropositives were obtained from pigs that had bats roost around/close to the farm while 32.8% of seropositives were obtained for pigs that did not have bats close to the farm. There was statistically significant association between prevalence and presence of bat around the farm ($P < 0.05$) (Table 1).

Table 3: Risk factors associated with the prevalence of *Henipavirus* in horses in Zaria and environs.

Risk factor	Number	Number of seropositives (%)	Chi, df	*P* value	Odds ratio	95% CI
Management system						
Intensive	128	27 (21.1)	8.494, 1	0.0036	4.50	1.50–14
Semi-intensive	72	4 (5.5)				
Animal purpose						
Sport	106	27 (25.5)	17.172	0.0002	6.2	1.4–27
Leisure/traditional	56	2 (3.6)			1.5	0.20–11.1
Security	38	2 (5.3)			Ref.	
Source of water						
Well	33	1 (3)	4.692, 1	0.0303	0.14	0.02–1.1
Pipe-borne	167	30 (17.9)				
Feed type						
Homemade	88	6 (6.8)	9.043, 1	0.0026	0.25	0.10–0.65
Commercial	112	25 (22.3)				
Feeding method						
In the open	41	3 (7.3)	2.637, 1	0.1044	0.37	0.11–1.3
In the pen	159	28 (17.6)				
Bat presence						
No	59	3 (5.1)	22.4	<0.0085	4.63	1.35–15.9
Yes	141	28 (19.8)				
Total	200	31 (15.5%)				

3.2. Horses. Horses in the <5 yrs category had the highest seroprevalence of 18.1% compared to 5–15 yrs and >15 yrs categories, both having seroprevalence of 15.2% and 14.8%, respectively (Table 4). There was no significant association between the presence of antibodies to *Henipavirus* in horses sampled and the sex of the horse ($P < 0.05$). Female horses had a higher seroprevalence of 19.8% than the male horses, 11% (Table 4). Out of the 200 horses sampled 100, 98, and 2 where local, exotic, and cross breeds, respectively. Breed seroprevalence of 6%, 25%, and 0% was obtained for local, exotic, and cross breed, respectively (Table 4).

Horses sampled were either imported ($n = 106$) or locally sourced ($n = 94$) with a seroprevalence of 23.5% and 6.3%, respectively. The purpose for keeping horse in Zaria and environs was for sport ($n = 106$), leisure/tradition ($n = 56$), and security ($n = 38$). These categories had a seroprevalence of 25.4%, 3.5%, and 5.2%, respectively. The sources of water available for the horse were well ($n = 33$) and pipe-borne ($n = 167$) water with seroprevalence of 3% and 17.9%, respectively. There was a statistically significant difference in seropositivity between the sources of water consumed by the horse (Table 3). The location specific prevalence obtained was 6.3%, 0%, 24.7%, and 7.4% for Zaria, Sabon-Gari, Igabi, and Kaduna North, respectively (Table 3).

A total of 122 horses were fed commercial feed and 88 consumed homemade feed. There was a statistically significant difference in feed type with an individual prevalence of 6.8% and 22.1% for homemade and commercial feed, respectively ($P < 0.05$) (Table 3). A seroprevalence of 7.3% and 17.6 was observed for horses fed in the open or in the pen in seropositivity between the feeding methods (Table 3). Only one horse had its feed stored in the open out of the 200 horses sampled. The management system practiced was semi-intensive ($n = 72$) or intensive ($n = 128$). There was a statistically significant difference in seropositivity between the management practices. Semi-intensive practice had a seroprevalence of 5.5% while intensive practice had seroprevalence of 21% (Table 3).

A total of 141 horses had bats around the stable while 59 did not. The prevalence of antibodies was 19.8% for those with bats and 5.1% for those with no bats. There was a significant difference between the presence of bat and the presence of antibodies reactive to *Henipavirus* ($P < 0.05$) (Table 3).

4. Discussion

The findings of this study showed that antibodies to *Henipavirus* are present in sera of horses and pigs in Zaria and environs with a prevalence of 15.5% and 20%, respectively. This prevalence using *HeV* sG iELISA was lower than what was obtained by Hayman and his colleagues [23], who observed a prevalence of 39% and 22% for Nipah and Hendra, respectively, in *E. helvum* using Luminex binding assay. This study also investigated horses and pigs unlike most others who investigated only the major reservoir hosts. This indicates that infection with henipaviruses occur in Nigerian horses and pigs. These animals may constitute an important source of spread of the virus.

Breed et al. [22] also recorded a higher prevalence of 50% for *Henipavirus* in Papua New Guinea where 66 bats caught from three (3) different locations were screened. He also employed Luminex binding assay for initial screening after which they subjected the positive samples to viral neutralization test for confirmation.

Table 4: Seroprevalence of antibodies reactive to *Henipavirus* sG antigen in horses sampled in Zaria and environs based on age, sex, breed, and location.

Variables	Number (%)	Number of seropositive animals (%)	χ^2	*P* value	Odds ratio	95% CI
Age						
<5 years	22	4 (18.1)	0.297	0.944	1.24	0.38–3.98
5–15 years	151	23 (15.2)			Ref	
>15 years	27	4 (14.8)			0.97	0.31–3.06
Sex						
Male	99	11 (11)	2.883, 1	0.0895	0.51	0.23–1.12
Female	101	20 (19.8)				
Breed						
Local	100	6 (6)			0.19	0.07–0.48
Exotic	98	25 (25.5)	*14.879	<0.0001	Ref.	
Cross	2	0 (0)			0.58	0.03–12.42
Location						
Zaria	63	4 (6.3)	*12.461, 3	0.004	0.02	0.01–0.67
Sabon-Gari	9	0 (0)			0.18	0.01–2.81
Igabi	101	25 (24.7)			Ref	
Kaduna North	27	2 (7.4)			0.24	0.05–1.10
Total	200	31 (15.5)				

*Fisher's exact test.

There is presently no commercial vaccine available for protection for henipaviruses in Africa and these animals are not routinely vaccinated against *Henipavirus*. Therefore, the demonstration of antibodies in sera of bats, horse, and pig is highly suggestive of natural exposure to the virus. The disparity in seroprevalences obtained between the two sampled animals (horses and pigs) might be due to the degree of susceptibility of each species.

Our finding on the age-specific seroprevalence is dissimilar with that of Peel et al. [21], where most adult bats yielded seropositives. The higher seroprevalence observed in younger animals (horse <5 years; 18.1% and pigs <6 months; 21.3%), though not significant, is suggestive that young animals still have circulating maternal antibody. The female animals had a higher prevalence (Table 2 and Table 4) probably because more female animals are kept for reproductive purposes with just few males kept for servicing numerous females. This argues with the findings of Plowright et al [24] that observed that females are at significant higher risk of infection in flying fox than males especially during pregnancy and lactation. Female animals are retained in their numbers for longer periods than males making the virus more established in them due to infection and reinfection. In fact in this study area, more female horses are kept even for sporting purposes.

Exotic breeds probably had higher seroprevalence (horse, 25.5%; pig, 55%) because they were not immunologically strong to handle the infection as they are continuously embattled with new infections and environmental stress. The local breeds exhibited a hardy immune system.

Animals that are not indigenous are not immunologically potent to handle and curtail disease(s). This is probably the reason why imported horses (from Argentina) and pigs (from Holland) had higher prevalence than local breeds. The imported animals battle different stressors ranging from environmental stress to exposure to new infectious agents to which their immunity has not been pruned. It is possible that these imported animals actually came with *Henipavirus* infection since this virus is not being screened for at all.

Giwa and Igabi have sparsely distributed population with abundant trees and vegetation. This provides a natural and undisturbed habitat for the bat reservoir host and a probable continual dissemination of the virus in the environment.

Animals watered with pipe-borne water had a higher seroprevalence (pig, 31.4%; horse, 17.9%). This could be due to contamination by bats reservoir host, inefficient water treatment, fomite, and poor sanitary condition as pipe-borne water in this environment has its origin from dammed water bodies that bats drink from and also contaminate. The higher seroprevalence observed in animals fed commercial feed could be attributed to insufficient biosecurity practices in feed milling facility and also within the farm. This impaired biosecurity protocol allows the continuous spread of the virus within the farm and also to other farms especially during clearing sales. The bat reservoir host could also contaminate feed when stored in large quantities.

Animal fed in the pen had a higher seroprevalence, though not statistically significance. This could be because the bat reservoir host gains access to the pen thereby contaminating the feeding trough. Animals that had their feed stored in the feed house probably had the feed contaminated by the bat reservoir host which would normally gain access to feed houses.

The management system of the intermediate hosts may have contributed to the seropositivity recorded. Pigs and horses kept under intensive management are restricted and confined, giving room for close contact and cross contamination between animals. Pigs in intensive system are usually grouped depending on the programme on the farm (either

breeding or fattening). In this study, all pigs from institutional farm were kept under intensive management system. This allowed for ease in spread of the virus through contact with discharges, body fluids, and even fomites.

Horses and pigs under intensive management may have had a high seroprevalence (horse, 21.1%; pig, 58.1%) due to contamination of feed and feeding trough by urine or faeces of bats that gain access to the farm. The virus is thus maintained more in intensive system as animals share the same feed and water source that could be contaminated by the reservoir host. This agrees with the work of Pulliam et al. [25].

Horses used for sport and sport related activities had a higher seroprevalence ($P < 0.05$). This group of animals is involved in various sporting events like polo allowing them close contact and cross contamination with numerous horses from different parts of the country. During this energy consuming events, horses are even tethered overnight under tree where fruit bats feed. This allows for a direct contamination of horse feed and water with urine, faeces, and feed droplets from bats.

The presence or absence of bat around the pen in horses and pigs yielded significant differences ($P < 0.05$) with higher prevalence found in horses that had bat around their pen (19.8%) and higher prevalence among pigs that did not have bat roost around their pen. This is probably because of the gregarious and capricious feeding habit of pigs. This finding also further strengthens the ubiquitous nature of the reservoir host enhanced by flight such that even though not seen around, it could still transmit the virus to animals. Seroprevalence of *Henipavirus* among human populations, detecting and characterizing the virus, and examining its zoonotic potential are needed in the future.

5. Conclusion

There is an evidence of *Henipavirus* in Zaria, Kaduna state, Nigeria, with a species-specific seroprevalence of 15.5% and 20% of horses and pigs, respectively. Based on our results, the tested animals were likely infected with an unidentified *Henipavirus*. The prevalence has serious significant public health and economic implication on livestock owners, farmers, hunters, and professional health workers like veterinarians, veterinary assistants, and foresters.

Pigs managed under intensive management system, imported, given well water, fed homemade feed, and fed in the open without a feed storage plan are at the risk of *Henipavirus* infection. Horses managed under intensive system, used for sport and traditional purposes consume homemade feed, consume well water and feed in the open re at the risk of *Henipavirus* infection.

Demographically, local and exotic pigs and horses, pigs in Zaria and Sabon-Gari, and horses in Zaria, Sabon-Gari, and Kaduna North are associated with the seroprevalence of henipaviruses.

There is need for a nationwide surveillance to provide additional information on the epidemiology of *Henipavirus* in Nigerian horses and pigs. Pig and horse handlers should be enlightened on the importance of good sanitary and biosecurity measures.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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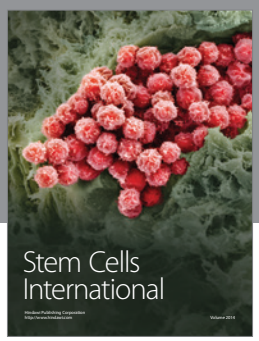

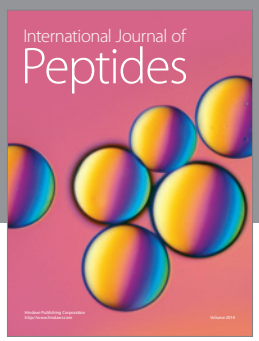

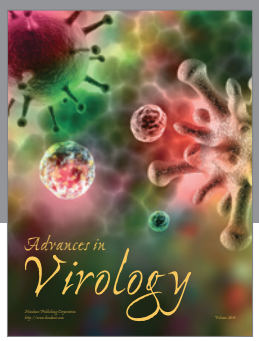

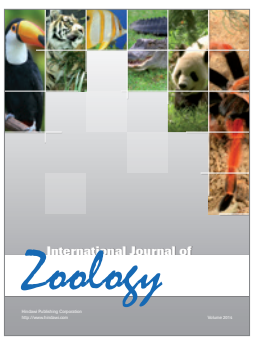

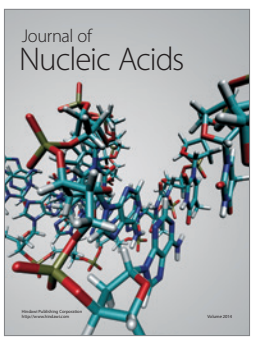

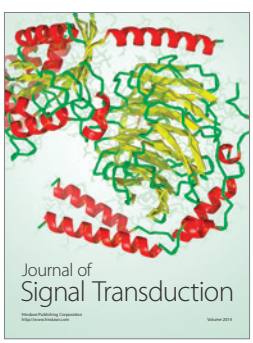

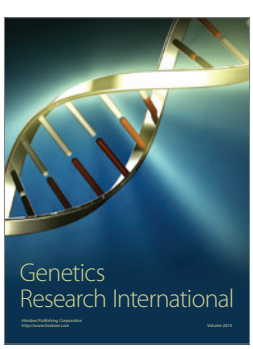

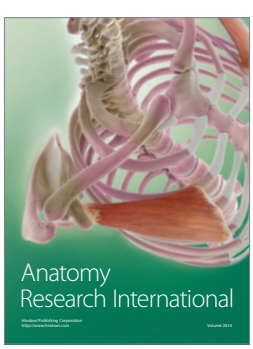

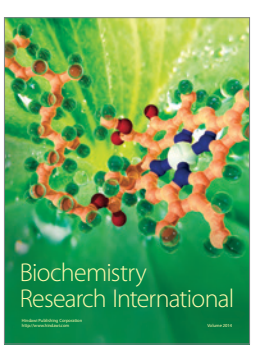

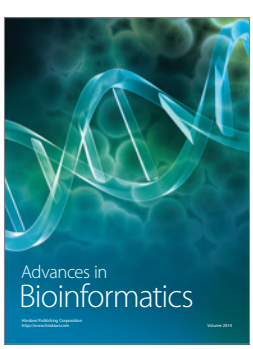

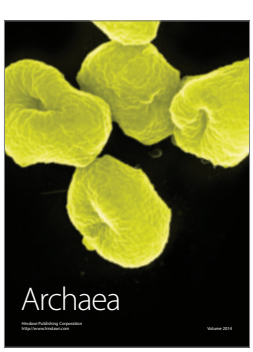

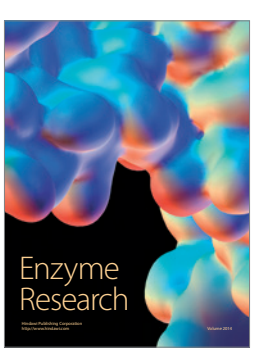

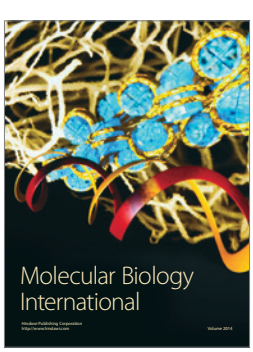

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