ANIMAL STUDIES RELATED TO BALKAN ENDEMIC NEPHROPATHY

B. Šoštarić, M. Peraica and R. Fuchs
Institute for Medical Research and Occupational Health University of Zagreb, Zagreb, Croatia

Received April 17, 1992.

Basic field studies related to the animal population were performed in the region of Slavonski Brod, Republic of Croatia, where balkan endemic nephropathy is an endemic disease. Pathological changes in several animal species from the locality were examined. The pig population in the area is numerous. Morphologically and physiologically pigs make an excellent animal model for studies of human diseases. Their use in studies should be encouraged, especially because there is a possibility that pigs and humans suffer from the same type of intoxication with a specific xenobiotic of natural origin. According to the mycotoxin theory about the etiology of Balkan endemic nephropathy, pork meat might be one of the possible hazards for humans. Experiments on laboratory animals provide an excellent possibility to monitor several aspects of pathogenesis and all stages of pathomorphological changes which might then be correlated with Balkan endemic nephropathy. However, the experimental species should be critically chosen because some spontaneous, species-specific lesions of the kidneys are easily mistaken for changes induced experimentally.

Key terms: animal population, endemic disease, field studies, rural area.

Despite an unknown etiology, different aspects of Balkan endemic nephropathy (BEN) have been studied very thoroughly, and are actually well described in a number of convincing papers. Probably the best known is the epidemiology of the disease, owing to extensive investigations performed in the endemic areas during a long period of time (1–4). Those areas with their topological, hydrological, pedological and climatic features have been well recognized and described. The epidemiological pictures concerning the populations from different endemic areas described by several independent groups of authors are generally comparable.

In this paper we shall first give a brief overview of our preliminary field studies and then discuss research on animals. The data are related to the well-known hyperendemic area of Slavonski Brod in the Republic of Croatia. The area is entirely rural. It is based on farm-type production, where each household owns a small piece of land and tends to be self-supporting. Traditionally, food supply comes almost exclusively from people’s own farming. People grow cereals, mostly corn for animal feed and wheat for own consumption. The area is comparatively rich. Corn was used as a foodstuff only during World War II and a few years after the war, but since then its use has by no means been extensive.
Generally, each household keeps several species of domestic animals. The area is rich in surface waters. The most frequent among the fowl are ducks and geese, which at least partially depend on own food supply from within the reach of a house. Dairy cattle is the commonest large animal. Years ago each household used to keep several of those animals, but today they are encountered less frequently. However, most households keep at least one. Horses, once very numerous, are rather scarce today (the area was one of the horse producing regions for marketing). Dog and cat populations are generally similar to those in the other rural areas, and are found in each house. Finally, there is a large number of pigs, which are the most important and most numerous domestic animals. They are farm-bred, and have been produced for several generations in the area. Actually, the area has been known for pork production; only a small amount of pork will be consumed by the farmer. Animal production is generally extensive, but the feeding and keeping conditions are rather traditional. There are still some villages, close to oak woods, where pigs are fed for a month or two almost exclusively on acorn crop. Whole herds will be left in a nearby wood, brought home afterwards, fattened with corn and slaughtered. During the sowingings period animals rarely get in contact with wild animals. Pigs are fed home-produced feed, which consists largely of corn, with the seasonal addition of pumpkins, potatoes and clover. In our experience, daily human table left-overs, although readily given to pigs, are not of any meaningful amount (a pound or two), because the farmer usually keeps more than five animals. There is a practice of producing larger quantities of corn than is necessary for one year, to prevent the consequences from possible failure of the next year’s harvest. The corn from the previous year than will be used first, the fact which might have certain implications if a mycotoxin is involved in the etiology of BEN. The pigs for the people’s own need are slaughtered when they reach the weight of 150-200 kg. As the farmers slaughter and process pigs in their own backyards, the meat is not subject to veterinary inspection, and many disease processes might be overlooked.

Finally, close to humans and their food mice and rats live inevitably, feeding on the same cereals as man and domestic animals, and frequently contaminating them with their excreta. Every late autumn the human habitats are invaded by fluctuant mice populations. The rats are of the sedentary type and belong to black rat (*Rattus rattus*). They mostly live in the attics. It seems that these commensalistic animals are no more numerous in endemic than in non-endemic (control) areas.

It is possible that some members of a certain species carry the same disease process as humans having BEN, if some xenobiotic deriving from the fields, or produced during the storage of grain is involved. Some of those animals, through their products, may enter the human nutritional chain, and thus get involved in the disease causation. During agricultural activities in the fields, farmers get indirectly in contact with several wild animal species, most notably with micromammals. A possibility of such contact was much more real a few decades ago, when for lack of machinery farmers spent much more time working on the land and were in closer contact with it.

In the proximity of agricultural fields large woods offer habitat to several wild animal species. Direct contact with animals from the ecosystem (through hunting and meat consumption) or an indirect one are relatively frequent. Since all the endemic areas are normally located in the immediate vicinity of rivers, the arthropod charge of the area is a very rich one, and cannot be neglected. As a matter of fact, some of the presently hyperendemic areas were foci of endemically occurring malaria many years ago.

The question of possible involvement in BEN of a species other than human has remained open until today. If the fact that some other animal species might suffer from
this disease is established, a reliable animal model for human disease could be made and studied. The aetiological agent could then be recognized enabling the prevention and control of human disease, which in the last three decades has already taken too many lives, and still seems to be entirely out of control. By analogy with biocenosis, where all species have to be considered as a unique, interrelated life phenomenon, observing the pathology of a certain human population during a given period of time without taking into consideration the pathocenoses of all possibly related species from the given area is artificial, and a definite conclusion about human pathology cannot be drawn.

The data gathered during the past 30 years of research indicate two main possibilities regarding the aetiological agent of BEN. One refers to a hypothetical infectious agent, mainly supported by the specific epidemiological picture of the disease, closely resembling the infectious disease of natural foci. The other theory advocates involvement of some toxin, but the researchers disagree as to the xenobiotic in question. With those two main pathways all proposed theories about the aetiology of BEN are by no means exhausted. However, we are not going to refer to all those theories where animal research is without possible implications, as it is for instance with the genetic theory. The followers of the infectious agent theory believe that the humans get infected during their agricultural activities through contact with wild animals, most probably micromammals. The hypothetical infectious agent could be a virus, slowly inducing a progressive tubular disease, finally resulting in specific clinical and morphological manifestations in the late phase of the disease. Some authors observed virus-like particles in the kidney material taken from patients with BEN (5), others used chickens as a possible model for coronavirus kidney infection (6, 7). However, there has been neither conclusive morphological nor virological support from studying human material favouring virus as aetiological agent of BEN. That fact does not exclude a possible virus implication, because at the late stages of the disease, when the tissues are usually examined, the virus will not necessarily be present. The possibility of some animal species acting as vector for the hypothetical viral agent has not been sufficiently examined, and larger, well conducted field animal investigations are called for. In order to obtain reliable information, several profiles of laboratory research including morphological, virological, bacteriological and some combined immunological techniques should be performed in a sufficient number of animals during a longer period of time. Speaking about a possible infectious agent contracted from micromammals to humans, one cannot avoid leptospires, the organisms so frequent in the renal areas, which are known to flourish in water ponds during the warm weather. It has to be mentioned, without any prejudice, that the lesions of chronic leptospirosis in dog kidneys, generally bacteriologically negative, remind a great deal of the kidney morphology in people having BEN. Years ago, researchers knowing that fact, investigated the incidence of leptospirosis in the human population in endemic areas obtaining positive results (8). Although the work is not conclusive, it will be interesting to compare the incidence and the strain of leptospirosis infection in wild as well as in commensalistic micromammals and domestic animals, especially pigs from the endemic and control areas. Once again we have to emphasize the need for long-term studies, because of the specific occurrence of infection, depending largely on the weather and the number of micromammals. Rozhajc and co-workers (9) performed a histopathological study of the wild rat kidney, and although certain pathological findings were stated, they are considered to be non-contributory to the problem resolution. The other theory, favouring a toxicological aetiology, is largely supported by epidemiological observation, this time interpreted as the disease of the houses, where the members of certain household are more frequently diseased, regardless of consanguinity. Lately, the accumulated evidences have indicated a possible role of
some mycotoxic agent in the aetiology of BEN. Definitely, one of the most strongly sugges-
ted xenobiotics has been ochratoxin A, a secondary metabolite of some moulds growing
as contaminants of grains, food and feed.

A progressive kidney disease of pigs, caused by ochratoxin A, has been reported from
different countries (10–13). Because of remarkable morphological and functional similarities
between porcine ochratoxicosis and BEN, it was postulated that both processes were
induced by ochratoxin A, and a possible animal model for BEN was established (14). In the
meantime, new evidences and supports have accumulated. A few studies (15–19) were
performed with the intention to demonstrate the morphological changes similar to human
nephropathy in domestic animals but without satisfactory results (20, 21). Taking into
consideration that the incidence of BEN among the human population of the hyperen-
demic villages is about 4 per cent, it might be supposed that the incidence among pigs
would not exceed this rate. Presumably, the works mentioned failed to demonstrate possible
changes because of a relatively small number of animals observed. Although the lifespan
of domestic pigs is much shorter in relative biological expectancy when compared with
humans, the lesions due to ochratoxin A intoxication have been demonstrated in many
thousands of farm-growing pigs of short lifespan. We therefore think that research on
pigs should be more extensive and should receive a great deal more encouragement. On
the other hand our knowledge of the changes in human kidneys at the early stage of
disease is relatively poor, which makes comparison with the changes observed on slaughter
pig kidneys even more complex. The positive correlation of heavy rainfalls with the in-
creased mortality of patients with BEN in certain years is interpreted by some authors as
the result of higher contamination of grains with mycotoxins (22). Thus, the failure to
demonstrate specific lesions in pig kidneys one year cannot be a decisive proof for excluding
the endemic aspect of intoxication, for it can occur next year, depending on weather
conditions as well as on specific way of grain storage. The results of several laboratory
animal studies related to ochratoxin A toxicity are in accordance with clinical findings
recognized in humans affected with BEN, such as the oncogenic properties (23) and sup-
pression of haemopoietic tissues (24–26). Although ochratoxin A is definitely the best known
and the most thoroughly investigated among nephrotoxic mycotoxins, possibly involved
in the aetiology of BEN, it is not the only one suspected. Pestanis and co-workers (27)
published an excellent paper on unidentified mycotoxin showing a potent nephrotoxic
activity. The strain of the *Penicillium* used in this experiment with rats was obtained from a
hyperendemic area in Bulgaria. Similar lesions on rat kidneys have been induced and
described recently (28). The *Penicillium* strain producing the toxin applied has been isolated
from corn collected in the endemic area near Slavonik Brod. The main lesions in both
experiments involved the proximal tubule epithelium which is known to be damaged in
patients with BEN. As there is strong evidence of synergistic effects among different
mycotoxins in laboratory animals (29), it is of major importance to investigate a possible
synergistic effect of all discovered mycotoxins from the area in question. Furthermore,
possible synergistic effects of mycotoxins and other nephrotoxic agents, some of which
may act as a non-specific initiating factor, must be investigated.

Based on the results of field investigations in domestic animals from endemic villages
and on experimental data the monitoring of pig kidney pathology from the endemic areas
should be conducted over a period of several years. Hundreds of samples from the hyper-
endemic region near Slavonik Brod and from the surroundings of Kosevsko Vitina (anot-
her endemic region) have been collected over two consecutive years. By comparison of
the results for each year, possible differences in the kidney pathology could be recognized.

Correlating BEN with some other, naturally occurring animal disease with a possible
value in further investigation, bovine enzootic haematuria cannot be avoided. Although
the kidney lesions due to that disease are not comparable with those caused by BEN in humans, the diseases have certain similarities (30). Bovine enzozoic haematuria is a very well-known bovine disease causing considerable economic losses and its strict enzozoic distribution has been recognized in several countries (31–33). The enzozoic areas of the disease do not overlap with the endemic nephropathy areas in former Yugoslavia. The suspected, though not definitely proved aetiology of bovine enzozoic haematuria is poisoning with a certain plant (bracken fern). A remarkable pathological feature of that disease is the incidence of urothelial tumours, quite comparable to those caused by BEN. It is interesting to note that urothelial tumours are characterised by the same histological appearance as those in BEN (32, 34). It will be necessary to perform some epidemiological studies as well as some analytical work in order to determine the presence of a possibly similar agent in both endemic and enzozoic areas inducing urothelial neoplasms. Considering the specific type of bovine digestion which is largely dependent on the microflora in their digestive tract, and their specific potentials of detoxification, it might be that the same agent is involved.

Regardless of the investigated animal species deriving from the endemic area, and the material and methodology used, some general principles should be respected in all further investigations, which will facilitate the use of data, and approach to a definite answer. Crucial for success in such type of work is establishment of a bank, where all material, including fixed and frozen tissue specimens will be kept for an indefinite period of time, available for all consecutive analytical procedures and comparison of results. Should some as far unknown evidence of a possible aetiological agent appear, the material could be analysed in that direction. An interdisciplinary scientific approach is imperative. The financial basis will thus be better focussed, enabling the processing of a larger number of samples, offering more precise answers.

Our considerations on the role of animals in the studies related to BEN cannot, of course, be completed without stressing the importance of experiments on laboratory animals. Essentially, there is no suspected agent which is not involved in some type of experiment on animals in laboratory conditions. A paper has been published (35) encouraging researchers in the use of laboratory animals. The author performed quite ingenious experiments with rats giving them concentrated water from the endemic area in order to discover a possible mineral xenobiotic (36). Marković (37) compared the guinea pig kidney lesions previously treated with silicates with morphological findings in BEN. Macanović (38) informed about possible immunological aspects of BEN, when the process takes place in the kidney interstitium.

A guinea pig animal model (39) has been registered for the study of human kidney disease, where an immunologically induced basal membrane disorder is the underlying aetiology. We advocate the use of this animal model in further research, rather than rat models because rats are prone to develop degenerative kidney lesions as a spontaneous age-related disease (40), which is easily confused with the effects of some xenobiotic tested. A number of papers refer to different experimental animal species as a model to study specific problems concerning BEN (41–43). The other aspects of investigation imply the use of animals living in the proximity of inhabitants in the BEN region. No less attention should be paid to the species living free in the area. Such field studies being rather scarce more efforts should be concentrated in that direction.
REFERENCES


Sažetak

ISTRAŽIVANJA NA ŽIVOTINJAMA U VEZI S BALKANSKOM ENDEMSKOM NEFROPATIJOM

Obavljena su osnovna terenska ispitivanja životinjskih populacija u okolici Slavonskog Broda, u kojoj je balkanska endemska nefropatija endemska bolest, i ispitane su patološke promjene na različitim životinjskim vrstama. Populacija svinja u promatranom području vrlo je brojna, a zbog svoje morfološke i fiziološke sličnosti čovjeka svinja je odličan model za bolesti ljudi. Ireira poticati studije na svinjama, osobito zbog mogućnosti da su svinje i ljudi izloženi intoxikaciji istim ksenobiotikom prirodnog podrijetla. U skladu s mikotoksičnom teorijom o etiologiji balkanske endemske nefropatije, svinjsko meso moglo bi biti opasno za čovjeka. Pokušaj na životinjama pružaju odličnu mogućnost promatranja različitih vidova patogeneze i patomorfoloških promjena u svim stadijima razvoja, koji lako mogu biti usporedivani s balkanskim endemskom nefropatijom.Ipak, eksperimentalne vrste moraju biti kritički izabrane, jer neke spontane lezije rubrega specifične za pojedine vrste mogu biti protumačene kao one izazvane eksperimentalno.

Institut za medicinska istraživanja i medicinu rada Sveučilišta u Zagrebu, Zagreb, Hrvatska

Ključne riječi: endemska bolest, ruralno područje, terenska ispitivanja, životinjska populacija.